EFFECT OF DRUGS ON THE LETHALITY IN MICE OF THE VENOMS AND NEUROTOXINS FROM SUNDRY SNAKES

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ABSTRACT

R. D. CROSLAND. Effect of several drugs on the lethality in mice of venoms and neurotoxins from sundry snakes, *Toxicon* , 19 . I investigated the efficacy of ten drugs with respect to reducing the lethality in mice of some or all of the following venoms and their respective neurotoxins: *Bungarus caeruleus* venom, *Bungarus multicinctus* venom and α-bungarotoxin and β-bungarotoxin, *Crotalus durissus terrificus* venom and crotoxin, *Notechis scutatus scutatus* venom, and *Oxyuranus scutellatus* venom and taipoxin. Venom or toxin was administered i.p., followed immediately by an i.p. injection of drug. The effect of the drug on the lethality of the venom or toxin was recorded 24 hr later. Diltiazem, nicergoline, primaquine, verapamil, and vesamicol protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom, and/or β-bungarotoxin. Dexamethasone provided protection from *B. multicinctus* venom, β-bungarotoxin, crotoxin, *O. scutellatus* venom, and taipoxin. Protective activity best correlated with the charge of the drug at physiological pH. Protection from lethality was maximal when the drugs were administered immediately after the injection of venom or toxin. Nifedipine, piracetam, reserpine, and vesamicol analog 72 provided no protection from any of the venoms/toxins tested.

INTRODUCTION

Antivenoms are the pharmacological agents currently used for treatment of intoxication due to snake venoms. Several factors, however, limit their usefulness. A given antivenom is effective against the venoms from only a small number of species of snakes, necessitating the availability of several antivenoms, and requiring the victim or physician to identify the guilty snake, which in many cases cannot be done. Furthermore, some people are hypersensitive to antivenoms. Finally, antivenoms require refrigeration, are sometimes needed in large quantities, and are expensive --- three factors that limit their availability. Treatment of snake venom intoxication would be greatly enhanced if a drug could be found which would overcome these deficiencies of antivenoms.

Some snake venoms contain presynaptic toxins which constitute the most lethal components of the venoms (CHANG, 1985). These presynaptic toxins act by inhibiting the release of acetylcholine from neurons, thereby blocking muscle contraction, which results in respiratory failure and death. These toxins have Ca^{2+}-dependent phosphatidate 2-acylhydrolase (EC 3.1.1.4) (trivial name: phospholipase A_2) activity, which is implicated in their toxicity (CHANG, 1985). Venoms which contain such presynaptic toxins include those from the snakes *Bungarus caeruleus* (Indian krait), *Bungarus multicinctus* (many-banded krait), *Crotalus durissus terrificus* (South American rattlesnake), *Notechis scutatus scutatus* (Eastern tiger snake), and *Oxyuranus scutellatus* (taipan) (common names are from ROSENBERG, 1987). Some snake venoms also contain postsynaptic toxins (*e.g.* α-bungarotoxin) which work in concert with presynaptic toxins by binding to the acetylcholine receptor and also blocking muscle contraction.

I previously reported (CROSLAND, 1988; 1989a) that chloroquine, chlorpromazine, and quinacrine were effective antagonists of the lethality in mice of *B. caeruleus* venom, *B. multicinctus* venom, and the latter's presynaptic toxin, β-bungarotoxin. A salient feature of these drugs is their ability to inhibit phospholipase A_2 activity (AUTHI and TRAYNOR, 1979; JAIN and JAHAGIRDAR, 1985; BROEKMEIER *et al.*, 1985). Other drugs which inhibit phospholipase A_2 activity may be more efficacious or have a wider spectrum of action as antagonists of snake venom lethality than those drugs tested heretofore. An additional class of drugs which merits investigation is the Ca^{2+} antagonists. These drugs antagonize many

Ca^{2+}-dependent processes (ORTEGA *et al.*, 1987; RADDINO *et al.*, 1987; ZERNIG, 1990) and could inhibit the Ca^{2+}-dependent phospholipase A_2 activity and thus the toxicity of snake presynaptic toxins. Also of interest are reserpine and vesamicol, which inhibit transport of neurotransmitters into synaptic vesicles. ANDERSON *et al.* (1983) reported that chloroquine, chlorpromazine, quinacrine, reserpine, and vesamicol inhibited transport of acetylcholine into synaptic vesicles from the electric organ of *Torpedo californica*. Since I found chloroquine, chlorpromazine, and quinacrine to be effective antagonists of snake venoms, reserpine and vesamicol could be also.

MATERIALS AND METHODS

Materials

Bungarus caeruleus venom, *B. multicinctus* venom, *C. durissus terrificus* venom, *O. scutellatus* venom, α-bungarotoxin, β-bungarotoxin, and crotoxin were purchased from Miami Serpentarium Laboratories, Salt Lake City, UT, U.S.A. *N. scutatus scutatus* venom and taipoxin were purchased from Ventoxin Laboratories, Frederick, MD, U.S A. Lyophilized venoms and toxins (except *C. durissus terrificus* venom and crotoxin) were dissolved (1 mg/ml) in deionized water. *C. durissus terrificus* venom was dissolved (0.5 mg/ml) in 20 mM sodium phosphate, pH 7.4. Crotoxin was dissolved (1 mg/ml) in 10 mM sodium chloride + 10 mM sodium acetate. Venom and toxin solutions were stored in aliquots at -20° C and were not refrozen after thawing. On the day of the experiment, venoms and toxins were further diluted with gel-phosphate buffer (0.2% gelatin (w/v), 0.4% sodium phosphate (w/v), pH 6.2). Dexamethasone (9-fluoro-11β,17,21-trihydroxy-16α-methylpregna-1,4-diene-3,20-dione) 21-phosphate (disodium salt), diltiazem (cis-(+)-3-(acetyloxy)-5-[2-(dimethylamino)ethyl]-2,3-dihydro-2-(4-methoxyphenyl)-1,5-benzothiazepin-4(5H)-one) hydrochloride, nifedipine (1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl-3,5-pyridinedicarboxylic acid dimethyl ester), primaquine (8-[4-amino-1-methylbutylamino]-6-methoxyquinoline) diphosphate, reserpine (11,17α-dimethoxy-18β-[(3,4,5-trimethoxybenzoyl)oxy]-3β,20α-yohimban-16β-carboxylic acid methyl ester), and verapamil (α-[3-[[2-(3,4-dimethoxyphenyl)ethyl]-methylamino]propyl]-3,4-dimethoxy-α-(1-methylethyl)benzeneacetonitrile) hydrochloride were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Nicergoline (10-methoxy-1,6-dimethylergoline-8β-methanol 5-bromonicotinate) was the gift of Farmitalia Carlo Erba, Milan, Italy. Piracetam (2-oxo-1-pyrrolidineacetamide) was a gift from Dr. Harvey Altman of the Lafayette Clinic, Detroit, MI, U.S.A. Vesamicol [(±)-2-(4-phenylpiperidino)cyclohexanol] hydrochloride was purchased from Research Biochemicals, Inc., Natick, MA, U.S.A. Vesamicol analog 72 [(±)-*trans* -5-amino-2-hydroxy-3-(4-phenylpiperidino)tetralin] was the gift of Dr. Stanley Parsons, University of California, Santa Barbara, CA, U.S.A. Chloroquine, chlorpromazine,

dexamethasone, diltiazem, piracetam, primaquine, and quinacrine were dissolved in 150 mM sodium chloride, 6 mM sodium phosphate (pH 7.2) (phosphate-buffered saline). Reserpine, nifedipine, and vesamicol analog 72 were dissolved in dimethylsulfoxide and then diluted 1-> 50 with polyethylene glycol:water::1:1 (vol). Verapamil was dissolved in water. Nicergoline was dissolved in a given volume of 25 mM tartaric acid, diluted with 1.13 volumes of water, followed by addition of 0.45 volume of 25 mM sodium bicarbonate. The final pH was 4-5. Vesamicol was dissolved in 6.25 mM tartaric acid (pH 3.7). The appropriate vehicle without dissolved drug was the control for each experiment.

Methods

Female ICR mice (20-30 g; Harlan Sprague-Dawley, Inc., Frederick, MD, U.S.A.) were housed five per cage, maintained on a 12 hr light-dark (1800 - 0600) cycle, and allowed free access to food and water. The mice were injected i.p.with the venom or toxin of interest in gel-phosphate buffer, followed by an i.p. injection of either drug solution or control solution.. All doses are expressed per kg mouse, were adjusted for the weight of the animal, and were administered in a volume of 10 ml/kg. The number of animals that died within 24 hr of the time of injection of venom or toxin was used as the measure of lethality.

The effect of various doses of a particular drug was tested by injecting mice with approximately two times the LD_{50} of the venom or toxin of interest, immediately followed by a separate injection of the drug. If the drug provided significant protection from the venom or toxin, then further investigation of the drug's interaction with that venom or toxin was pursued. The ED_{50} and the LD_{50} of the dose-effect experiments refer to those doses of drug required to produce 50% of the maximal observed protective effect and were calculated by using the data of the rising and falling phases, respectively, of the dose-effect curve. Please note that the LD_{50} of the drug was determined in the presence of venom or toxin, and was not the LD_{50} of the drug alone.

The optimal time of injection of a drug was determined by injecting the most protective dose of the drug at different times before (-60, -30, -15 min) or after (+0, +15, +30, +60 min) the injection of approximately two times the LD_{50} of the venom or toxin of interest. Control animals received an injection of venom or toxin which was either preceeded (-45 min) by an injection of vehicle alone (one-half of controls) or followed (+45 min) by an injection of vehicle alone (one-half of controls).

Each experiment was repeated at least once, and the data were combined. Each data point represents at least five mice. A p value associated with a change in the LD_{50} of a venom or toxin due to drug treatment refers to the drug's effect on the dose-response curve as calculated using logit analysis. Other tests of significance were calculated using contingency or regression analysis. Statistical tests were considered significant when $p < 0.05$.

RESULTS

Dexamethasone

Dexamethasone protected mice from the lethality of *B. multicinctus venom*, β-bungarotoxin, crotoxin, *O. scutellatus* venom and taipoxin; while providing no protection from *B. caeruleus* venom, *C. durissus terrificus* venom, α-bungarotoxin, or *N. scutatus scutatus* venom (Fig. 1). In the cases of *B. multicinctus venom*, β-bungarotoxin, and crotoxin, protection increased with increasing doses of dexamethasone (the optimal doses were 15 μmol/kg, 75 μmol/kg, and 6.2 μmol/kg, respectively) and then declined with further increasing doses. With *O. scutellatus* venom and taipoxin, however, protection increased with increasing doses of dexamethasone (100% protection at 90 μmol/kg and 60 μmol/kg, respectively) and remained at 100% with further increases in dosage. The ED_{50}s of dexamethasone were 5.8 μmol/kg, 7.2 μmol/kg, 0.17 μmol/kg, 51 μmol/kg, and 22 μmol/kg with respect to *B. multicinctus venom*, β-bungarotoxin, crotoxin, *O. scutellatus* venom, and taipoxin. The LD_{50}s with respect to *B. multicinctus* venom, β-bungarotoxin and crotoxin were 44 μmol/kg, 87 μmol/kg, and 30 μmol/kg; while the corresponding therapeutic indices were 7.6, 12 and 176. There was no declining phase to the remaining dose-response curves, so no LD_{50}s or therapeutic indices could be calculated for them.

Dexamethasone increased the LD_{50} of *O. scutellatus* venom 3.5-fold from 22 μg/kg to 76 μg/kg ($p < .0005$) (Fig. 2). It completely protected mice from a dose of venom that was lethal to 86% of the untreated mice. Dexamethasone also increased the LD_{50} of taipoxin 4.0-fold from 2.5 μg/kg to 10 μg/kg ($p = .001$), completely protecting mice from a dose of the toxin that was lethal to all of the untreated mice. At 66 μmol/kg, dexamethasone had no significant effect, however, on the LD_{50} of *B. multicinctus* venom, increasing it from 70 μg/kg to 120 μg/kg ($p = 0.10$) (data not shown); or at 6.2 μmol/kg, on the LD_{50} of crotoxin, increasing it from 58 μg/kg to 90 μg/kg ($p = 0.88$) (data not shown). An injection of gel-phosphate buffer followed immediately by an injection of 90 μmol/kg of dexamethasone made 20 mice lethargic and sleepy for several hr. After 24 hr all of the mice recovered.

Dexamethasone exhibited maximal protective action when it was administered to mice immediately following intoxication with *O. scutellatus* venom or taipoxin (0 min, Fig. 3). In

the case of *O. scutellatus* venom, moreover, protection was evident as long as 30 min after intoxication, whereas no such post intoxication protection was observed with taipoxin.

Diltiazem

Diltiazem protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin, while providing no protection from α-bungarotoxin, *C. durissus terrificus* venom, crotoxin, *N. scutatus scutatus* venom, *O. scutellatus* venom, or taipoxin (Fig. 4). Protection increased with increasing amounts of diltiazem up to 5.5 μmol/kg in the case of *B. caeruleus* venom, 22 μmol/kg in the case of *B. multicinctus* venom, and 11 μmol/kg in the case of β-bungarotoxin. Higher doses of diltiazem resulted in a decline in effectiveness. The ED_{50}s of diltiazem with respect to *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin were 2.4 μmol/kg, 4.1 μmol/kg, and 2.0 μmol/kg, respectively. The LD_{50}s were 31 μmol/kg, 89 μmol/kg, and 72 μmol/kg, resulting in therapeutic indices of 13, 21, and 36.

Diltiazem increased the LD_{50} of *B. caeruleus* venom 2.2-fold from 51 μg/kg to 110 μg/kg ($p = 0.010$) (Fig. 5). It also increased the LD_{50} of *B. multicinctus* venom 7.4-fold from 23 μg/kg to 170 μg/kg ($p = 0.0010$). In fact, it completely protected mice from two times a lethal dose of *B. multicinctus* venom. Diltiazem also increased the LD_{50} of β-bungarotoxin 1.9-fold from 19 μg/kg to 37 μg/kg ($p = 0.014$). An injection of gel-phosphate buffer followed immediately by an injection of 22 μmol/kg of diltiazem caused no overt effects in 20 mice.

I investigated the effect of injecting diltiazem at different time intervals both before and after the injection of *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Diltiazem provided maximal protection in all three cases when it was injected immediately after (0 min) the injection of venom or toxin (Fig. 6). In the case of *B. caeruleus* venom, 15 min post administration of diltiazem provided some protection, while 15 min pre administration of diltiazem provided some protection from *B. multicinctus* venom.

Nicergoline

Nicergoline protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus*

venom, and β-bungarotoxin, while providing no protection from *C. durissus terrificus* venom, crotoxin, *O. scutellatus* venom, or taipoxin (Fig. 7). Protection increased with increasing amounts of nicergoline up to 8.3 μmol/kg in all cases. Higher doses of nicergoline resulted in a decline in effectiveness. The ED_{50} of nicergoline was 2.2 μmol/kg, 1.8 μmol/kg, or 1.9 μmol/kg with respect to *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Combining these values with LD_{50}s of 71 μmol/kg, 33 μmol/kg, and 76 μmol/kg resulted in therapeutic indices of 32, 18, and 40 for *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin, respectively.

I tested nicergoline (8.3 μmol/kg) for its ability to increase the LD_{50} of *B. caeruleus* venom, *B. multicinctus* venom, α-bungarotoxin, and β-bungarotoxin. It increased the LD_{50} of *B. multicinctus* venom 4.6-fold from 24 μg/kg to 110 μg/kg ($p < .0005$) and the LD_{50} of β-bungarotoxin 4.0-fold from 9.6 μg/kg to 38 μg/kg ($p < .0005$) (Fig. 8). It had no significant effect, however, on the LD_{50} of *B. caeruleus* venom or α-bungarotoxin, increasing the former's LD_{50} by 1.8-fold from 35 μg/kg to 62 μg/kg ($p = .073$) (data not shown) and increasing the latter's LD_{50} by 1.0-fold from 200 μg/kg to 210 μg/kg ($p = .39$) (data not shown). An injection of gel-phosphate buffer followed immediately by an injection of 8.3 μmol/kg of nicergoline had no overt effect on 20 mice observed for 48 hr.

I investigated the effect of injecting nicergoline at different time intervals both before and after the injection of *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Nicergoline provided maximal protection in all three cases when it was injected immediately after (0 min) the injection of venom or toxin (Fig. 9). In no case did pre administration or post administration (other than 0 min) of nicergoline afford mice protection from intoxication.

Nifedipine

Nifedipine did not protect mice from the lethality of any of the venoms or toxins tested (Fig. 10), and no further investigation of its interaction with them was pursued. An injection of gel-phosphate buffer followed immediately by an injection of 29 μmol/kg of nifedipine had no overt effect on 20 mice observed for 24 hr.

Piracetam

Piracetam failed to protect mice from the lethality of any of the venoms or toxins tested (Fig. 11), and no further investigation of its effects on them was undertaken. An injection of gel-phosphate buffer followed immediately by an injection of 7,000 µmol/kg of piracetam had no overt effect on 20 mice observed for 24 hr.

Primaquine

Primaquine protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin while providing no protection from *C. durissus terrificus* venom, crotoxin, *O. scutellatus* venom, or taipoxin (Fig. 12). Protection increased with increasing amounts of primaquine up to 44 µmol/kg in the case of *B. caeruleus* venom, 11 µmol/kg in the case of *B. multicinctus* venom, and 22 µmol/kg in the case of β-bungarotoxin. Higher doses of primaquine resulted in a decline in effectiveness. The ED_{50} of primaquine was 2.1 µmol/kg, 1.9 µmol/kg, or 7.2 µmol/kg with respect to *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Combining these values with LD_{50}s of 98 µmol/kg, 78 µmol/kg, and 49 µmol/kg resulted in therapeutic indices of 47, 41, and 6.8 for *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin, respectively.

Primaquine increased the LD_{50} of *B. caeruleus* venom 2.9-fold from 27 µg/kg to 79 µg/kg ($p < .0005$), of *B. multicinctus* venom 6.0-fold from 35 µg/kg to 210 µg/kg ($p < .0005$), and of β-bungarotoxin 3.9-fold from 8.8 µg/kg to 34 µg/kg ($p = 0.002$) (Fig. 13). In fact, primaquine completely protected mice from a dose (about three times the LD_{50}) of *B. multicinctus* venom that killed 100% of the control mice. Primaquine (11 µmol/kg) had no significant effect on the LD_{50} of α-bungarotoxin, increasing it by 1.2-fold from 320 µg/kg to 390 µg/kg ($p = .12$) (data not shown). An injection of gel-phosphate buffer followed immediately by an injection of 11 µmol/kg of primaquine had no overt effect on 20 mice observed for 48 hr.

I investigated the effect of injecting primaquine at different time intervals both before and after the injection of *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Primaquine provided maximal protection in all three cases when it was injected immediately

after (0 min) the injection of venom or toxin (Fig. 14). Fifteen minute pre administration of primaquine afforded protection from intoxication due to *B. multicinctus* venom or β-bungarotoxin. Primaquine also provided protection from *B. multicinctus* venom or β-bungarotoxin at 15 min post intoxication.

Reserpine

Reserpine did not protect mice from the lethality of any of the venoms or toxins tested (Fig. 15), and no further investigation of its interaction with them was pursued. An injection of gel-phosphate buffer followed immediately by an injection of 5.1 µmol/kg of reserpine sedated 20 mice for 24 hr. All of the mice recovered after 48 hr.

Verapamil

Verapamil protected mice from the lethality of *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin, while providing no protection from α-bungarotoxin, *C. durissus terrificus* venom, crotoxin, *N. scutatus scutatus* venom, *O. scutellatus* venom, or taipoxin (Fig. 16). Protection increased with increasing amounts of verapamil up to 5.1 µmol/kg in all cases. Higher doses of verapamil resulted in a decline in effectiveness. The ED_{50}s of verapamil with respect to *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin were 1.5 µmol/kg, 1.0 µmol/kg, and 1.4 µmol/kg, respectively. The LD_{50}s were 51 µmol/kg, 53 µmol/kg, and 17 µmol/kg, respectively, resulting in therapeutic indices of 34, 53, and 12.

Verapamil (10.2 µmol/kg) increased the LD_{50} of *B. caeruleus* venom 5.2-fold from 21 µg/kg to 110 µg/kg ($p = 0.001$) (Fig. 17). It provided almost complete protection from 30 µg/kg *B. caeruleus* venom, a lethal dose. Verapamil (5.1 µmol/kg) also increased the LD_{50} of *B. multicinctus* venom 3.8-fold from 36 µg/kg to 135 µg/kg ($p = 0.001$) and increased the LD_{50} of β-bungarotoxin 5.0-fold from 30 µg/kg to 150 µg/kg ($p = 0.001$). An injection of gel-phosphate buffer followed immediately by an injection of 5.1 µmol/kg of verapamil caused no overt effects in 20 mice observed for 24 hr.

I investigated the effect of injecting verapamil at different time intervals both before and after the injection of *B. caeruleus* venom, *B. multicinctus* venom, or β-bungarotoxin. Verapamil provided maximal protection in all three cases when it was injected immediately after

(0 min) the injection of venom or toxin (Fig. 18). It was ineffective when it was injected at other times.

Vesamicol

Vesamicol protected mice from the lethality of *B. caeruleus* venom and *B. multicinctus* venom, while providing no protection from α-bungarotoxin, β-bungarotoxin, *C. durissus terrificus* venom, crotoxin, *N. scutatus scutatus* venom, *O. scutellatus* venom, or taipoxin (Fig. 19). I observed protection over a broad range of doses, especially in the case of *B. caeruleus* venom. This might have been due to the presence of both entantiomers of vesamicol. The "optimal" dose of vesamicol was 4.2 μmol/kg in the case of *B. caeruleus* venom and 0.066 μmol/kg in the case of *B. multicinctus* venom. Higher doses of vesamicol resulted in a decline in effectiveness. The ED_{50}s of vesamicol with respect to *B. caeruleus* venom and *B. multicinctus* venom were 0.045 μmol/kg, and 0.027 μmol/kg, respectively. The LD_{50}s were 7.7 μmol/kg, and 0.93 μmol/kg, respectively, resulting in therapeutic indices of 171 and 34. Vesamicol analog 72 (0.0000011 μmol/kg to 3.4 μmol/kg) provided no protection from β-bungarotoxin (data not shown) and was not tested with any other venoms or toxins.

Vesamicol had no effect on the LD_{50} of *B. caeruleus* venom or *B. multicinctus* venom. At 0.0011 μmol/kg, it increased the LD_{50} of *B. caeruleus* venom 1.5-fold from 28 μg/kg to 42 μg/kg ($p = 0.49$), and at 0.26 μmol/kg it increased the LD_{50} of *B. multicinctus* venom 2.0-fold from 18 μg/kg to 36 μg/kg ($p = 0.13$). An injection of gel-phosphate buffer followed immediately by an injection of 4.2 μmol/kg of vesamicol caused no overt effects in 20 mice observed for 24 hr.

DISCUSSION

Table 1 summarizes the effects of twelve drugs on the lethality of venoms and neurotoxins from selected snakes. Examination of the table reveals interesting patterns in both the venoms/toxins and the drugs.

With the exception of dexamethasone, all of the effective drugs were so only against *B. caeruleus* venom, *B. multicinctus* venom, and β-bungarotoxin. In addition, (again with the exception of dexamethasone) any drug which was ineffective against *B. caeruleus* venom was also ineffective against *B. multicinctus* venom and β-bungarotoxin. I summarized these observations using two methods of correlation. As a qualitative correlation of the drugs' effects on the lethality of *B. multicinctus* venom *vs.* the drugs' effects on the lethality of β-bungarotoxin, I utilized Spearman's rank-order method (corrected for ties), using a value of one to represent a significant drug effect and a value of zero to represent no drug effect (ρ = 0.82, p = 0.0068). (If vesamicol were considered an effective drug against β-bungarotoxin [p = 0.059, Fig. 19], the correlation would be 1.00.) As a quantitative correlation I used Pearson's product-moment method on the relationship between the drug's effects on the LD_{50} of *B. multicinctus* venom *vs.* the drugs' effects on the LD_{50} of β-bungarotoxin (Fig. 20a) (r = 0.47, p = 0.15). (I chose to utilize the fold change in LD_{50} caused by a drug as the quantitative measure of efficacy because the fold change has no upper limit. For drugs which had no significant effect on lethality in the dose-effect experiments I assigned a fold change in LD_{50} of 1.0) The significant qualitative correlation demonstrates that a drug which protected mice from β-bungarotoxin was likely to protect mice from *B. multicinctus* venom. The non-significance of the quantitative correlation was due to the substantial change in the LD_{50} of β-bungarotoxin (17 fold) caused by chloroquine. Excluding this value raises the quantitative correlation to 0.79 (p = 0.0060). It would appear, therefore, that a drug's effect on the lethality of β-bungarotoxin was reflected in its effect on the lethality of *B. multicinctus* venom. This is not surprising since β-bungarotoxin is the most lethal component of *B. multicinctus* venom and contributes the majority of the venom's lethality (CHANG, 1985). Any drug which reduces

the lethality of β-bungarotoxin would be expected to reduce the lethality of *B. multicinctus* venom.

Correlations similar to those between the drugs' effects on β-bungarotoxin and *B. multicinctus* venom were observed between the drugs' effects on *B. caeruleus* venom and *B. multicinctus* venom. The qualitative correlation was 0.75 ($p = 0.012$) while the quantitative correlation was 0.31 ($p = 0.32$) (Fig. 20b). *B. caeruleus* venom not only comes from a snake of the same genus as *B. multicinctus*, it also contains presynaptic toxins with potencies similar to that of β-bungarotoxin (ABE *et al.*, 1977; LEE *et al.*, 1976). Although the relative contribution of these neurotoxins to the lethality of the whole venom has not been thoroughly studied, it would seem reasonable that this contribution is similar to that of β-bungarotoxin's contribution to the lethality of *B. multicinctus* venom. Thus, we could expect that any drug which reduces the lethality of *B. multicinctus* venom would also reduce the lethality of *B. caeruleus* venom. This, indeed, appears to be the case.

The same drugs (except dexamethasone) which protected mice from the two *Bungarus* venoms and β-bungarotoxin did not protect mice from *C. durissus terrificus* venom or its presynaptic toxin crotoxin, or *O. scutellatus* venom or its presynaptic toxin taipoxin. β-Bungarotoxin, crotoxin, and taipoxin have similar effects on neuromuscular transmission, have phospholipase A_2 activity, and are thought to act through similar biochemical mechanisms (CHANG, 1985). With respect to the action of the majority of the drugs tested, however, β-bungarotoxin was quite distinct from crotoxin and taipoxin. The discriminatory action of the drugs may be related to one or more of the salient differences among the toxins. β-Bungarotoxin, for example, consists of two polypeptide chains linked by a disulfide bond, whereas crotoxin and taipoxin are composed of two and three subunits, respectively. Also, β-bungarotoxin has a basic isoelectric point (9.1) (OTHMAN *et al.*, 1982), while both crotoxin and taipoxin have an acidic isoelectric point (5.0) (KARLSSON, 1979). The difference in isoelectric points, however, may not account entirely for the differential action of the drugs because all of them were ineffective against the lethality of α-bungarotoxin, which has an isoelectric point of 9.2 (ELDEFRAWI and FERTUCK, 1974). Finally, there is evidence that

β-bungarotoxin, crotoxin, and taipoxin bind at different sites on the presynaptic membrane (CHANG and SU, 1980; REHM and BETZ, 1982). The drugs may act to inhibit differentially the binding of the toxins, thus providing selective protection from the toxins and their respective venoms. Whatever the cause, the differential effect of the drugs on the lethality of the three toxins is further evidence of distinctions among the toxins.

Several observations can be made concerning the drugs that I used in these studies. One is that all but vesamicol have been used clinically in humans (BARNHART, 1989; BILLUPS and BILLUPS, 1989). Also, the effective drugs were generally so in doses which approximated those used clinically, suggesting that they acted through a clinically relevant mechanism. The effective agents did not, however, belong to a single therapeutic group of drugs, precluding correlation of venom/toxin antagonism with the primary therapeutic action of an agent. Diltiazem, nicergoline, nifedipine, and verapamil are vasodilators, and all but nifedipine were effective antagonists of the lethality of the *Bungarus* venoms and β-bungarotoxin. All of the antimalarial drugs tested --- chloroquine, primaquine, and quinacrine --- were likewise effective against the *Bungarus* venoms and β-bungarotoxin. Also effective in varying degrees were the tranquilizer chlorpromazine, the antiinflamatory agent dexamethasone, and the non-clinically utilized acetylcholine transport inhibitor vesamicol. The cerebral stimulant piracetam and the antihypertensive reserpine were ineffective.

On another level, diltiazem, nifedipine, and verapamil are Ca^{2+} antagonist drugs which could act to inhibit presynaptic toxin-related, Ca^{2+}-dependent phospholipase A_2 activity and consequently the lethality of the venoms/toxins (CHANG, 1985). Diltiazem and verapamil did, indeed, inhibit the lethality of the *Bungarus* venoms and β-bungarotoxin. Nifedipine, however, did not. From this admittedly limited sample, it did not appear that being a Ca^{2+} antagonist drug guaranteed effectiveness against the venoms/toxins.

Chloroquine, chlorpromazine, quinacrine, reserpine, vesamicol, and vesamicol analog 72 are inhibitors of acetylcholine transport into synaptic vesicles prepared from the electric organ of *Torpedo californica* (ANDERSON *et al.*, 1983; ROGERS *et al.*, 1989) (Table 2). Since chloroquine, chlorpromazine, and quinacrine were shown to be antagonists of the lethality of the *Bungarus* venoms and β-bungarotoxin (CROSLAND, 1988; 1989a; 1989b),

other transport inhibitors could have been also. The results, however, suggested otherwise because the fold changes in the LD_{50} of β-bungarotoxin due to the above drugs did not correlate (r = -0.33, p = 0.52) with their IC_{50}s with respect to acetylcholine transport, implying that their antagonism of the lethality of β-bungarotoxin was not related to their acetylcholine transport inhibitory activity.

The initial criterion for choosing a drug for this series of studies was that it inhibit phospholipase A_2 activity. The investigated venoms/toxins have phospholipase A_2 activity which has been implicated in their toxicity (CHANG, 1985), suggesting that inhibitors of this activity could reduce that toxicity. Two immediate problems with this hypothesis, however, are the observations that nifedipine and piracetam were ineffective against the lethality of any of the venoms/toxins and that all of the drugs (except dexamethasone) which provided protection from the *Bungarus* venoms and β-bungarotoxin were completely ineffective against *C. durissus terrificus* venom, crotoxin, *O. scutellatus* venom, and taipoxin. In the latter instance it is possible, though unlikely, that the effective drugs acted by inhibiting a *Bungarus* -specific phospholipase A_2 activity. *Apropos* of this possibility, there was a significant correlation (r = 0.73, p = 0.016) between the fold change in LD_{50} of *B. multicinctus* venom and the K_is of the drugs with respect to phospholipase A_2 activity (Fig. 21). A large part of this correlation, however, was contributed by the 11-fold increase in LD_{50} caused by quinacrine. Removal of this value from consideration reduced the correlation to 0.32 (p = 0.39), which was similar to that (r = 0.39, p = 0.26) between the fold change in the LD_{50} of *B. caeruleus* venom and K_i, and the correlation (r = 0.51, p = 0.16) between the fold change in the LD_{50} of β-bungarotoxin and K_i. It should also be noted that the correlations were positive. *A priori* I would expect that a phospholipase A_2 inhibitor with a low K_i would cause a large increase in LD_{50}, *i.e.*, the correlation would be negative. Moreover, an important *caveat* to this analysis is the determination of the K_is of the drugs with respect to phospholipase A_2 activity. The values that I used (Table 2) were the lowest that I found in the literature and were determined from assays that used different sources of phospholipase A_2, different substrates, and different detection methods. The pitfalls of comparing results from different studies of phospholipase A_2 activity have been well documented, particularly the problem of using non-physiological substrates (CHANG, 1985; ROSENBERG,1979). Unfortunately, due to their small relative mass, it is not possible to detect any

β-bungarotoxin-stimulated phosholipid hydrolysis at the presynaptic terminals of the phrenic nerve-diaphragm (GHASSEMI *et al.*, 1988), making it impossible to compare directly the relevant anti-phospholipase A_2 activity of the drugs with their protective activity. From the preponderance of the available data, however, I cannot conclude that the phospholipase A_2 inhibitory activity of the tested drugs was a significant factor in their ability to afford protection from the lethality of the venoms/toxins.

I examined the quantitative relationship between the protective ability of a drug and its molecular weight, solubility, and charge. There was no correlation between either the molecular weight o he solubility of a drug in phosphate-buffered saline and the fold change in LD_{50} for either of the *Bungarus* venoms or β-bungarotoxin (Table 3). There was, however, for both venoms and β-bungarotoxin a significant correlation between the protective ability of a drug and its positive charge at pH 7.2. Figure 22 illustrates this relationship for *B.. multicinctus* venom. (Dexamethasone was the only drug with a negative charge and was omitted from the correlation.) It appears that a drug needed a positive charge in order to antagonize the lethality of the venoms or β-bungarotoxin. Since the charge of a molecule is a major factor in its ability to bind to a receptor or enzyme, the successful antagonists may compete with the presynaptic toxins for binding to a receptor or they may compete with some substrate for binding to the toxins or for binding to some enzyme activated by the toxins. Whatever the mechanism may be, it seems to be limited to the *Bungarus* venoms and β-bungarotoxin.

Dexamethasone was the exceptional drug throughout this study. It was the only drug which was an effective antagonist of the lethality of venoms/toxins other than those from the *Bungarus* snakes (Table 1). It antagonized five of the nine venoms/toxins tested and almost antagonized α-bungarotoxin and *B. caeruleus* venom (Fig. 1). It was particularly effective (100%) against *O. scutellatus* venom and taipoxin, with which it did not exhibit declining effectiveness at high doses of drug (the only effective drug and venom/toxin combinations not to do so). Dexamethasone was also the only drug in this study to have a negative charge (-1.5) at pH 7.2 (due to the phosphate group attached to the parent molecule), also making it the only drug without a positive charge to antagonize effectively the lethality of *B. multicinctus* venom and

β-bungarotoxin. This could mean that any charge will serve to antagonize the venoms/toxin or that some other characteristic(s) of dexamethasone overcame the lack of a positive charge. The K_i (1 μM) of dexamethasone with respect to phospholipase A_2 activity was the second lowest of all the drugs tested. Molecular weight and solubility were in the midrange of the group.

Others have reported mixed results when corticosteroids were used to treat envenomation by snakes. BENYAJATI *et al.* (1961) found that prednisolone significantly enhanced the survival of dogs that had been injected with *Naja tripudians* (cobra) venom. They also found that cortisol or prednisolone was very beneficial in the treatment of three known and three presumed cobra-bite victims. REID (1964), on the other hand, reported that prednisone was not beneficial in the cases of four humans bitten by cobras (*Naja naja*). In addition, REID *et al.* (1963) reported that prednisone had no beneficial action on human envenomation by Malayan vipers (*Agkistrodon rhodostoma*). Although the above results and my results with dexamethasone are not rigorously comparable, considered *in toto* they suggest that corticosteroids or derivatives thereof could provide protection from the lethal venoms of several species of snakes.

The time of injection of a drug relative to the time of injection of the venom/toxin was an important factor in the drug's efficacy. All of the time-tested drugs were maximally effective when they were injected immediately after the venom/toxin was injected. Injection of the drug either 15 min before or 15 min after injection of the venom/toxin greatly reduced or, in some cases, eliminated effectiveness. None of the drugs was effective when it was injected 30 min prior to the injection of venom/toxin, and only chloroquine (CROSLAND, 1989b) and dexamethasone were even partially effective when they were injected 30 min after the injection of venom/toxin. The requirement for temporal proximity of injection of drug and venom/toxin may suggest that the drugs were protecting mice from the lethality of the venoms/toxins by interrupting some initial step(s) in intoxication such as transport and/or binding to the target organ.

The drugs utilized in this study can be grouped into three categories: drugs which antagonized the lethality of only the *Bungarus* venoms and β-bungarotoxin, dexamethasone, and drugs which were ineffective. All of the effective drugs carried a charge at physiological pH. Future research to delineate the properties of a drug which make it an antagonist of the lethality of snake venoms and toxins could lead to the development of drugs which are even

more effective and have a broader spectrum of action than those investigated to date.

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REFERENCES

ABE, T., ALEMA, S. and MILEDI, R. (1977) Isolation and characterization of presynaptically acting neurotoxins from the venom of *Bungarus* snakes. *Eur. J. Biochem.* **80**, 1-12.

ANDERSON, D. C., KING, S. C., and PARSONS, S. M. (1983) Pharmacological characterization of the acetylcholine transport system in purified *Torpedo* electric organ synaptic vesicles. *Molec. Pharmacol.* **24**, 48-54.

AUTHI, K. S. and TRAYNOR, J. R. (1979) Effects of antimalarial drugs on phospholipase A_2. *Br. J. Pharmacol.* **66**, 496P.

BARNHART, E. R. (1989) *Physician's Desk Reference*, 43rd Edn., Oradell, NJ: Medical Economics.

BENYAJATI, C., KEOPLUNG, M., and SRIBHIBHADH, R. (1961) Experimental and clinical studies on glucocorticoids in cobra envenomation. *J. Trop. Med. Hyg.* **64**, 46-49.

BILLUPS, N.F. and BILLUPS, S.M. (1989) *American Drug Index*, St. Louis: Lippincott.

BROEKEMEIER, K. M., SCHMID, P. C., SCHMID, H. H. O., and PFEIFFER, D. R. (1985) Effects of phospholipase A_2 inhibitors on ruthenium red-induced Ca^{2+} release from mitochondria. *J. biol. Chem.* **260**, 105-113.

CHANG, C. C. (1985) Neurotoxins with phospholipase A_2 activity in snake venoms. *Proc. natl sci Counc. B. ROC* **9**, 126-142.

CHANG, C. C. and SU, M. J. (1980) Mutual potentiation, at nerve terminals, between toxins from snake venoms which contain phospholipase A activity: β-bungarotoxin, crotoxin, taipoxin. *Toxicon* **18**, 641-648.

CHANG, J., BLAZEK, E., and CARLSON, R. P. (1987) Inhibition of phospholipase A_2 (PLA_2) activity by nifedipine and nisoldipine is independent of their calcium channel-blocking activity. *Inflamation* **11**, 353-364.

CROSLAND, R. D. (1988) Effect of chloroquine on toxicity in mice of the venom and neurotoxins from the snake *Bungarus multicinctus*. *J. Pharmacol. exp. Ther.* **246**, 992-995.

CROSLAND, R. D. (1989a) Effect of chlorpromazine and quinacrine on the lethality in mice of the venoms and neurotoxins from several snakes. *Toxicon* **27**, 655-663.

CROSLAND, R. D. (1989b) Development of drug therapies for snake venom intoxication. In *Natural Toxins: Characterization, Pharmacology, and Therapeutics*, pp. 165-175 (Ownby, C. I.

and Odell, G. V., Eds.), Oxford: Pergamon Press.

ELDEFRAWI, M. E. and FERTUCK, H. C. (1974) A rapid method for the preparation of [^{125}I]α-bungarotoxin. *Anal. Biochem.*, **58**, 63-70.

GHASSEMI, A., DHILLON, D. S., and ROSENBERG, P. (1988) β-Bungarotoxin-induced phospholipid hydrolysis in rat brain synaptosomes: effect of replacement of calcium by strontium. *Toxicon* **26**, 509-514.

JAIN, M. K. and JAHAGIRDAR, D. V. (1985) Action of phospholipase A_2 on bilayers: effect of inhibitors. *Biochim. biophys. Acta* **814**, 319-326.

KARLSSON, E. (1979) Chemistry of protein toxins in snake venoms. *Handbook Exp. Pharmacol.* **52**, 157-212.

LEE, C. Y., CHEN, Y. M., and MEBS, D. (1976) Chromatographic separation of the venom of *Bungarus caeruleus* and pharmacological characterization of its components. *Toxicon* **14**, 451-457.

NIKOLOV, R. and KOBUROVA, K. (1984) Inhibition of phospholipase A2 *in vitro* by some anti-hypoxic drugs. *Meth. Find. Exptl. Clinc. Pharmacol.* **6**, 429-431.

ORTEGA, M. P., SUNKEL, C., PRIEGO, J. G., and STATKOW, P. R. (1987) The antithrombogenic *in vivo* effects of calcium channel blockers in experimental thrombosis in mice. *Thrombosis Haemostasis* **57**, 283-285.

OTHMAN, I. B., SPOKES, J. W., and DOLLY, J. O. (1982) Preparation of neurotoxic [^{3}H]β-bungarotoxin: demonstration of saturable binding to brain synapses and its inhibition by toxin I. *Eur. J. Biochem.*, **128**, 267-276.

PERRIN, D. D. (1965) *Dissociation Constants of Organic Bases in Aqueous Solution*, London:Butterworths

PERRIN, D. D. (1972) *Dissociation Constants of Organic Bases in Aqueous Solution: Supplement 1972*, London:Butterworths

PERRIN, D. D., DEMPSEY, B., and SERJEANT, E. P. (1981) *pK_a Prediction for Organic Acids and Bases*, London: Chapman and Hall.

PILTCH, A., SUN, L., FAVA, R., and HAYASHI, J. (1989) Lipocortin-independent effect of dexamethasone on phospholipase activity in a thymic epithelial cell line. *Biochem. J.* **261**, 395-400.

RADDINO, R., POLI, E., FERRARI, R., and VISIOLI, O. (1987) Effects of calcium entry

blockers not connecteed with calcium channels inhibition. *Gen. Pharmacol.* **18**, 431-436.

REHM, H. and BETZ, H. (1982) Binding of β-bungarotoxin to synaptic membrane fractions of chick brain. *J. biol. Chem.* **257**, 10015-10022.

REID, H. A. (1964) Cobra-bites *Br. Med. J.* **2**, 540-545.

REID, H. A., THEAN, P. E., and MARTIN, W. J. (1963) Specific antivenene and prednisone in viper-bite poisoning: controlled trial. *Br. Med. J.* **2**, 1378-1380.

ROGERS, G. A., PARSONS, S. M., ANDERSON, D. C., NILSSON, L. M., BAHR, B. A., KORNREICH, W. D. KAUFMAN, R., JACOBS, R. S., and KIRTMAN, B. (1989) Synthesis, in vitro acetylcholine-storage-blocking activities, and biological properties of derivatives and analogues of *trans* -2-(4-phenylpiperidino)cyclohexanol (vesamicol). *J. Med. Chem.* **32**, 1217-1230.

ROSENBERG, P. (1979) Pharmacology of phospholipase A_2 from snake venoms. *Handbook Exp. Pharmacol.* **52**, 401-447.

ROSENBERG, P. (1987) Common names index. *Toxicon* **25**, 800-890.

ZERNIG, G. (1990) Widening potential for Ca^{2+} antagonists: non-L-type Ca^{2+} channel interaction. *Trends Pharmacol. Sci.* **11**, 38-44.

Legends for Tables

TABLE 1. SUMMARY OF EFFECTS OF DRUGS ON VENOMS AND TOXINS

Data for chloroquine, chlorpromazine, and quinacrine are from CROSLAND, 1989a; 1989b. Nifedipine, piracetam, and reserpine had no effect on any of the venoms/toxins tested.

na = not applicable.

nd = not determined.

ns = not significant.

TABLE 2. PROPERTIES OF DRUGS

(a) The charge of a drug was calculated from the measured pK_a(s) when available (PERRIN, 1965; PERRIN, 1972). When the measured pK_a(s) of a drug was not available, I estimated it by using the methods in PERRIN *et al.* (1981).

(b) Values from ANDERSON *et al.* (1983) and ROGERS *et al.* (1989).

(c) The solubility of a drug in phosphate-buffered saline was determined by diluting the drug in 2-fold steps from 1 g/ml to 0.125 mg/ml. The concentration (in molarity) at which the drug completely dissolved was taken as its solubility. Note that this value could have almost a 2-fold error. Nicergoline, nifedipine, and reserpine did not completely dissolve at 0.125 mg/ml, but I used this value to calculate their solubilities.

TABLE 3. CORRELATION (r) OF FOLD CHANGE IN LD_{50}S WITH DRUGS' PROPERTIES

(a) $p = 0.041$

(b) $p = 0.0068$

(c) $p = 0.014$

Legends for Figures

FIG. 1. DOSE-EFFECT OF DEXAMETHASONE ON THE LETHALITY OF VENOMS AND TOXINS

(a) Mice were injected with 80 µg/kg of *B. multicinctus* venom (●), 30 µg/kg of β-bungarotoxin (▲), 150 µg/kg of crotoxin (○), 50 µg/kg of *O. scutellatus* venom (△) or 5 µg/kg of taipoxin (+), followed immediately by a separate injection of various doses of dexamethasone. Test of overall significance, % of control mice surviving: *B. multicinctus* venom, 0.0001, 0%; β-bungarotoxin, 0.032, 17%; crotoxin, 0.038, 12%; *O. scutellatus* venom, 0.0011, 0%; taipoxin, 0.0001, 10%. (b) Mice were injected with 350 µg/kg α-bungarotoxin (X), 50 µg/kg of *B. caeruleus* venom (■), 200 µg/kg of *C. durissus terrificus* venom (❑), or 200 µg/kg of *N. scutatus scutatus* venom (❑), followed immediately by a separate injection of various doses of dexamethasone. Test of overall significance, % of control mice surviving: α-bungarotoxin, 0.084, 10%; *B. caeruleus* venom, 0.15, 10%; *C. durissus terrificus* venom, 0.73, 7%; *N. scutatus scutatus* venom, 0.82, 25%.

FIG. 2. EFFECT OF DEXAMETHASONE ON THE LD_{50} OF *O. SCUTELLATUS* VENOM AND TAIPOXIN.

Mice were injected with various amounts of *O. scutellatus* venom (△,▲) or taipoxin (○,●), followed immediately by a separate injection of either phosphate-buffered saline (empty symbols) or 90 µmol/kg dexamethasone (filled symbols).

FIG. 3. EFFECT OF RELATIVE TIME OF INJECTION OF DEXAMETHASONE ON THE LETHALITY OF *O. SCUTELLATUS* VENOM AND TAIPOXIN.

Mice were injected with 20 µg/kg of *O. scutellatus* venom (△) or 4 µg/kg of taipoxin (+), each preceeded (negative times) or followed (0 and positive times) by an injection of 90 µmol/kg of dexamethasone. Tests of overall significance, % of control mice surviving: *O. scutellatus*

venom, 0.0001, 5%; taipoxin, 0.0001, 15%.

FIG. 4. DOSE-EFFECT OF DILTIAZEM
ON THE LETHALITY OF VENOMS AND TOXINS

(a) Mice were injected with 80 µg/kg of *B. caeruleus* venom (■), 80 µg/kg of *B. multicinctus* venom (●), or 50 µg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of diltiazem. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0005, 5%; *B. multicinctus* venom, 0.0001, 0%; β-bungarotoxin, 0.0001, 13%. (b) Mice were injected with 350 µg/kg of α-bungarotoxin (X), 150 µg/kg of *C. durissus terrificus* venom (❑), 150 µg/kg of crotoxin (○), 200 µg/kg of *N. scutatus scutatus* venom (❑), 20 µg/kg of *O. scutellatus* venom (△), or 4 µg/kg of taipoxin (+) followed immediately by a separate injection of various doses of diltiazem. Test of overall significance, % of control mice surviving: α-bungarotoxin, 0.094, 10%; *C. durissus terrificus* venom, 0.60, 0%; crotoxin, 0.19, 0%; *N. scutatus scutatus* venom, 0.26, 0%; *O. scutellatus* venom, 0.39, 20%; taipoxin, 1.00, 0%.

FIG. 5. EFFECT OF DILTIAZEM ON THE LD_{50}
OF *BUNGARUS* VENOMS AND β-BUNGAROTOXIN.

Mice were injected with various amounts of venoms or toxin, followed immediately by a separate injection of either phosphate-buffered saline (empty symbols) or diltiazem (filled symbols). *B. caeruleus* venom, 11 µmol/kg diltiazem (❑,■); *B. multicinctus* venom, 22 µmol/kg diltiazem (○,●); β-bungarotoxin, 11 µmol/kg diltiazem (△,▲).

FIG. 6. EFFECT OF RELATIVE TIME OF INJECTION OF DILTIAZEM
ON THE LETHALITY OF *BUNGARUS* VENOMS AND

β-BUNGAROTOXIN.

Mice were injected with venoms or toxin, either preceeded (negative times) or followed (0 and positive times) by an injection of diltiazem. 70 µg/kg *B. caeruleus* venom, 11 µmol/kg diltiazem (■); 80 µg/kg *B. multicinctus* venom, 22 µmol/kg diltiazem (●); 50 µg/kg β-bungarotoxin, 11 µmol/kg diltiazem (▲). Tests of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0001, 10%; *B. multicinctus* venom, 0.0001, 0%; β-bungarotoxin, 0.0081, 10%.

FIG. 7. DOSE-EFFECT OF NICERGOLINE
ON THE LETHALITY OF VENOMS AND TOXINS

(a) Mice were injected with 50 µg/kg of *B. caeruleus* venom (■), 50 µg/kg of *B. multicinctus* venom (●), or 30 µg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of nicergoline. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0003, 20%; *B. multicinctus* venom, 0.0003, 20%; β-bungarotoxin, 0.0001, 0%. (b) Mice were injected with 200 µg/kg of *C. durissus terrificus* venom (❑), 100 µg/kg of crotoxin (○), 20 µg/kg of *O. scutellatus* venom (△), or 2 µg/kg of taipoxin (+), followed immediately by a separate injection of various doses of nicergoline. Test of overall significance, % of control mice surviving: *C. durissus terrificus* venom, 0.33, 0%; crotoxin, 0.40, 20%; *O. scutellatus* venom, 0.33, 0%; taipoxin, 0.71, 20%.

FIG. 8. EFFECT OF NICERGOLINE ON THE LD_{50}
OF *B. MULTICINCTUS* VENOM AND β-BUNGAROTOXIN.

Mice were injected with various amounts of *B. multicinctus* venom or β-bungarotoxin, followed immediately by a separate injection of either tartrate (empty symbols) or 8.3 µmol/kg nicergoline (filled symbols). *B. multicinctus* venom (○,●), β-bungarotoxin (△,▲).

FIG. 9. EFFECT OF RELATIVE TIME OF INJECTION OF NICERGOLINE ON THE LETHALITY OF *BUNGARUS* VENOMS AND β-BUNGAROTOXIN.

Mice were injected with 70 μg/kg of *B. caeruleus* venom (■), 50 μg/kg of *B. multicinctus* venom (●), or 25 μg/kg of β-bungarotoxin (▲), each preceeded (negative times) or followed (0 and positive times) by an injection of 8.3 μmol/kg of nicergoline. Tests of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0001, 0%; *B. multicinctus* venom, 0.0001, 17%; β-bungarotoxin, 0.0001, 3%.

FIG. 10. DOSE-EFFECT OF NIFEDIPINE ON THE LETHALITY OF VENOMS AND TOXINS

Mice were injected with 80 μg/kg of *B. caeruleus* venom (■), 60 μg/kg of *B. multicinctus* venom (●), 350 μg/kg of α-bungarotoxin (X), 25 μg/kg of β-bungarotoxin (▲), 150 μg/kg of *C. durissus terrificus* venom (❑), 150 μg/kg of crotoxin (○), 200 μg/kg of *N. scutatus scutatus* venom (❑), 20 μg/kg of *O. scutellatus* venom (△), or 2 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of nifedipine. Tests of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.19, 10%; *B. multicinctus* venom, 0.16, 0%; α-bungarotoxin, 0.070, 6.7%; β-bungarotoxin, 0.42, 33%; *C. durissus terrificus* venom, 0.42, 30%; crotoxin, 0.34, 10%; *N. scutatus scutatus* venom, 0.25, 20%; *O. scutellatus* venom, 0.71, 20%; taipoxin, 0.83, 30%.

FIG. 11. DOSE-EFFECT OF PIRACETAM ON THE LETHALITY OF VENOMS AND TOXINS

Mice were injected with 40 μg/kg of *B. caeruleus* venom (■), 75 μg/kg of *B. multicinctus* venom (●), 50 μg/kg of β-bungarotoxin (▲), 200 μg/kg of *C. durissus terrificus* venom (❑), 100 μg/kg of crotoxin (○), 20 μg/kg of *O. scutellatus* venom (△), or 2 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of piracetam. Tests of overall significance, % of control mice surviving: *B. caeruleus* venom 0.34, 0%; *B.*

multicinctus venom, 1.00, 0%; β-bungarotoxin 0.40, 0%; *C. durissus terrificus* venom, 0.89, 10%; crotoxin, 0.70, 25%; *O. scutellatus* venom, 0.34, 0%; taipoxin, 0.23, 10%.

FIG. 12. DOSE-EFFECT OF PRIMAQUINE ON THE LETHALITY OF VENOMS AND TOXINS

(a) Mice were injected with 50 µg/kg of *B. caeruleus* venom (■), 100 µg/kg of *B. multicinctus* venom (●), or 30 µg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of primaquine. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0006, 30%; *B. multicinctus* venom, 0.0066, 20%; β-bungarotoxin, 0.0001, 0%. (b) Mice were injected with 200 µg/kg of *C. durissus terrificus* venom (❑), 100 µg/kg of crotoxin (○), 20 µg/kg of *O. scutellatus* venom (△), or 2 µg/kg of taipoxin (+), followed immediately by a separate injection of various doses of primaquine. Test of overall significance, % of control mice surviving: *C. durissus terrificus* venom, 0.33, 0%; crotoxin, 0.14, 30%; *O. scutellatus* venom, 1.00, 0%; taipoxin, 0.26, 0%.

FIG. 13. EFFECT OF PRIMAQUINE ON THE LD_{50} OF *BUNGARUS* VENOMS AND β-BUNGAROTOXIN.

Mice were injected with various amounts of venoms or toxin, followed immediately by a separate injection of either phosphate-buffered saline (empty symbols) or primaquine (filled symbols). *B. caeruleus* venom, 44 µmol/kg primaquine (❑,■); *B. multicinctus* venom, 11 µmol/kg primaquine (○,●); β-bungarotoxin, 11 µmol/kg primaquine (△,▲).

FIG. 14. EFFECT OF RELATIVE TIME OF INJECTION OF PRIMAQUINE ON THE LETHALITY OF *BUNGARUS* VENOMS AND β-BUNGAROTOXIN.

Mice were injected with 40 µg/kg of *B. caeruleus* venom (■), 75 µg/kg of *B. multicinctus* venom (●), or 30 µg/kg of β-bungarotoxin (▲), each preceeded (negative times) or followed (0 and positive times) by an injection of 44 µmol/kg of primaquine (*B. caeruleus* venom) or 11

μmol/kg of primaquine (*B. multicinctus* venom and β-bungarotoxin). Tests of overall significance, % of control mice surviving: all cases, 0.0001, 0%.

FIG. 15. DOSE-EFFECT OF RESERPINE
ON THE LETHALITY OF VENOMS AND TOXINS

Mice were injected with 80 μg/kg of *B. caeruleus* venom (■), 60 μg/kg of *B. multicinctus* venom (●), 350 μg/kg of α-bungarotoxin (X), 30 μg/kg of β-bungarotoxin (▲), 150 μg/kg of *C. durissus terrificus* venom (❑), 150 μg/kg of crotoxin (○), 200 μg/kg of *N. scutatus scutatus* venom (❑), 20 μg/kg of *O. scutellatus* venom (△), or 4 μg/kg taipoxin (+), foliowed immediately by a separate injection of various doses of reserpine. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.20, 5%; *B. multicinctus* venom, 0.20, 5%; α-bungarotoxin, 0.65, 15%; β-bungarotoxin, 0.90, 10%; *C. durissus terrificus* venom, 0.51, 15%; crotoxin, 0.73, 15%; *N. scutatus scutatus* venom, 0.054, 0%; *O. scutellatus* venom, 0.81, 5%; taipoxin, 0.21, 0%.

FIG. 16. DOSE-EFFECT OF VERAPAMIL
ON THE LETHALITY OF VENOMS AND TOXINS

(a) Mice were injected with 60 μg/kg of *B. caeruleus* venom (■), 60 μg/kg of *B. multicinctus* venom (●), or 50 μg/kg of β-bungarotoxin (▲), followed immediately by a separate injection of various doses of verapamil. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0001, 7%; *B. multicinctus* venom, 0.0010, 20%; β-bungarotoxin, 0.0001, 0%. (b) Mice were injected with 350 μg/kg of α-bungarotoxin (X), 150 μg/kg of *C. durissus terrificus* venom (❑), 150 μg/kg of crotoxin (○), 200 μg/kg of *N. scutatus scutatus* venom (❑), 20 μg/kg of *O. scutellatus* venom (△), or 4 μg/kg of taipoxin (+), followed immediately by a separate injection of various doses of verapamil. Test of overall significance, % of control mice surviving: α-bungarotoxin, 0.68, 10%; *C. durissus terrificus* venom, 0.40, 20%; crotoxin, 0.24, 10%; *N. scutatus scutatus* venom, 0.74, 10%; *O.*

scutellatus venom, 0.59, 0%; taipoxin, 1.00, 0%.

FIG. 17. EFFECT OF VERAPAMIL ON THE LD_{50} OF *BUNGARUS* VENOMS AND β-BUNGAROTOXIN.

Mice were injected with various amounts of venoms or toxin, followed immediately by a separate injection of either water (empty symbols) or verapamil (filled symbols). *B. caeruleus* venom, 10.2 μmol/kg verapamil (❑,■); *B. multicinctus* venom, 5.1 μmol/kg verapamil (○,●); β-bungarotoxin, 5.1 μmol/kg verapamil (△,▲).

FIG. 18. EFFECT OF RELATIVE TIME OF INJECTION OF VERAPAMIL ON THE LETHALITY OF *BUNGARUS* VENOMS AND β-BUNGAROTOXIN.

Mice were injected with venoms or toxin, either preceeded (negative times) or followed (0 and positive times) by an injection of verapamil. 60 μg/kg *B. caeruleus* venom, 10.2 μmol/kg verapamil (■); 80 μg/kg *B. multicinctus* venom, 5.1 μmol/kg verapamil (●); 80 μg/kg β-bungarotoxin, 5.1 μmol/kg diltiazem (▲). Tests of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0001, 0%; *B. multicinctus* venom, 0.0001, 20%; β-bungarotoxin, 0.0001, 7%.

FIG. 19. DOSE-EFFECT OF VESAMICOL ON THE LETHALITY OF VENOMS AND TOXINS

(a) Mice were injected with 80 μg/kg of *B. caeruleus* venom (■) or 60 μg/kg of *B. multicinctus* venom (●), followed immediately by a separate injection of various doses of vesamicol. Test of overall significance, % of control mice surviving: *B. caeruleus* venom, 0.0005, 8%; *B. multicinctus* venom, 0.012, 2.5%. (b) Mice were injected with 350 μg/kg of α-bungarotoxin (X), 40 μg/kg of β-bungarotoxin (▲), 150 μg/kg of *C. durissus terrificus* venom (❑), 150 μg/kg of crotoxin (○), 200 μg/kg of *N. scutatus scutatus* venom (❑), 20 μg/kg of *O. scutellatus* venom (△), or 4 μg/kg taipoxin (+), followed immediately by a separate injection of various doses of vesamicol. Test of overall significance, % of control mice surviving:

α-bungarotoxin, 0.55, 10%; β-bungarotoxin, 0.059, 3.3%; *C. durissus terrificus* venom, 0.59, 20%; crotoxin, 0.65, 10%; *N. scutatus scutatus* venom, 0.69, 10%; *O. scutellatus* venom, 0.40, 20%; taipoxin, 1.00, 0%.

FIG. 20 CORRELATIONS OF FOLD CHANGES IN LD_{50}

(a) Plot of the drug-induced fold changes in the LD_{50} of β-bungarotoxin *vs.* the drug-induced fold changes in the LD_{50} of *B. multicintus* venom. (b) Plot of the drug-induced fold changes in the LD_{50} of *B. caeruleus venom vs.* the drug-induced fold changes in the LD_{50} of *B. multicintus* venom.

FIG. 21 CORRELATION OF FOLD CHANGES IN LD_{50} OF *B. MULTICINCTUS VENOM* AND K_i OF DRUGS TOWARD PHOSPHOLIPASE A_2

Plot of the drug-induced fold changes in the LD_{50} of *B. multicintus* venom *vs.* the inhibitory constants (K_i) of the drugs with respect to phospholipoase A_2 activity.

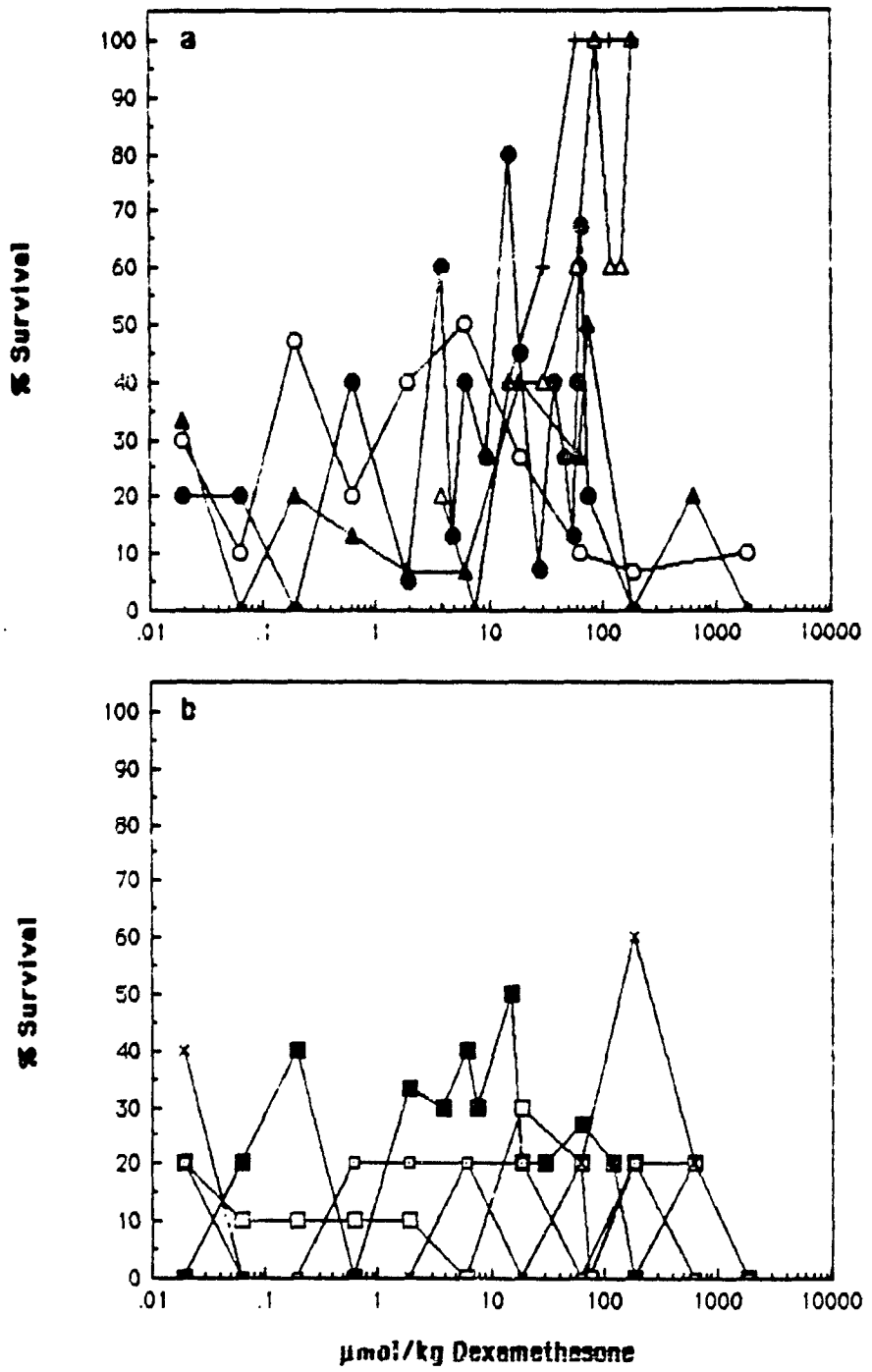
FIG. 22 CORRELATION OF FOLD CHANGES IN LD_{50} OF *B. MULTICINCTUS VENOM* AND CHARGE OF DRUGS AT pH 7.2

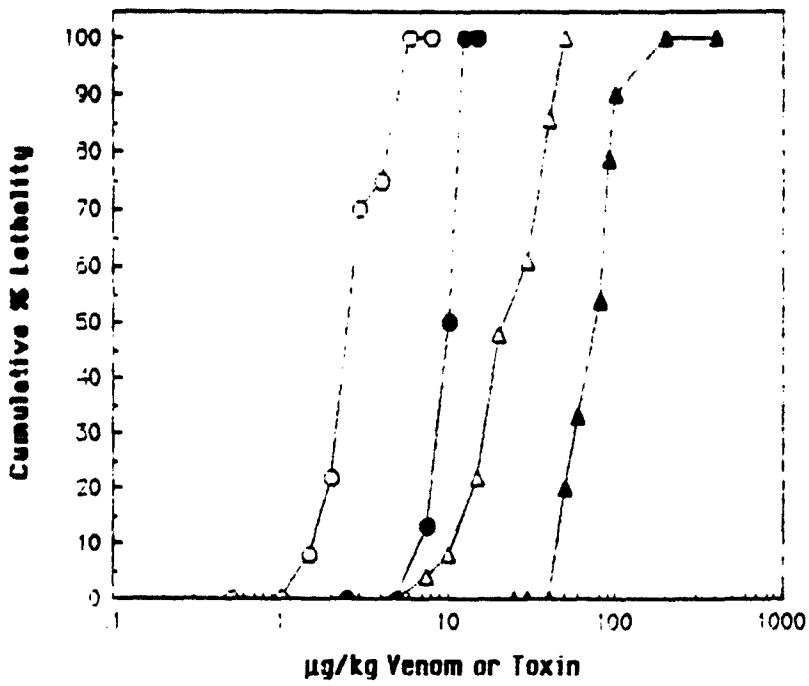
Plot of the drug-induced fold changes in the LD_{50} of *B. multicintus* venom *vs.* the charge on the drugs at pH 7.2

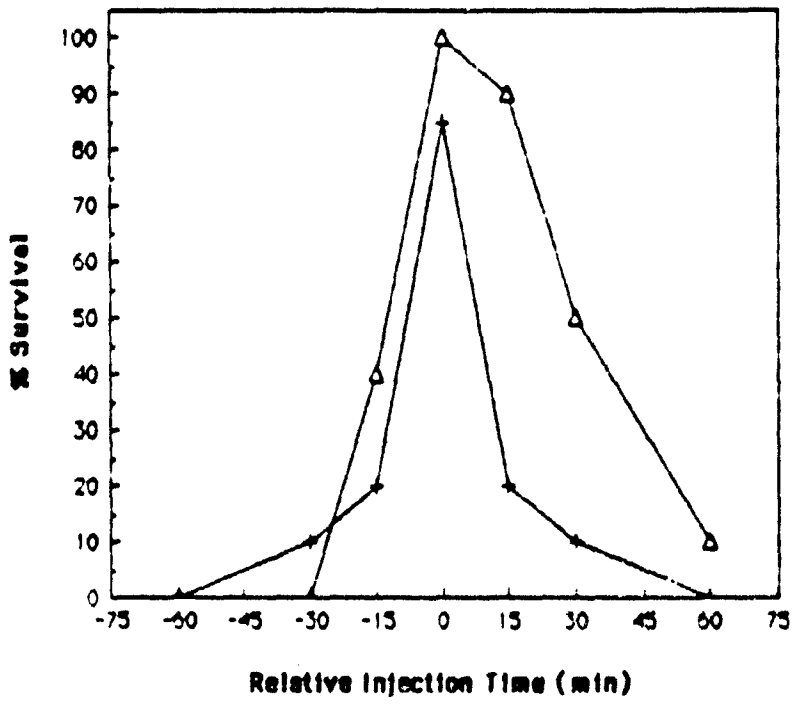
Parameter	Chlrqn	Chlrprmzn	Dxmthsn	Dltzm	Ncrgln	Prmqn	Qncrn	Vrpmil	Vsmol
Optimal Dose (µmol/kg)									
B. caeruleus venom	78	2.8	none	5.5	8.3	44	49	5.1	4.2
B. multicinctus venom	78	2.8	15	22	3.3	11	9.8	5.1	0.066
α-Bungarotoxin	none	none	none	none	nd	nd	none	none	none
β-Bungarotoxin	39	1.4	75	11	8.3	22	2.0	5.1	none
C. durissus venom	none	none	none	none	none	none	none	none	none
Crotoxin	none	none	6.2	none	none	none	none	none	none
N. scutatus venom	nd	nd	none	none	nd	nd	nd	none	none
O. scutellatus venom	none	none	90	none	none	none	none	none	none
Taipoxin	none	none	60	none	none	none	none	none	none
Maximal % Survival									
B. caeruleus venom	80	90	na	70	93	90	80	73	47
B. multicinctus venom	72	100	80	100	80	90	80	100	30
β-Bungarotoxin	100	100	50	87	100	100	100	100	na
C. durissus venom	na	na	na	na	na	na	na	na	na
Crotoxin	na	na	50	na	na	na	na	na	na
O. scutellatus venom	na	na	100	na	na	na	na	na	na
Taipoxin	na	na	100	na	na	na	na	na	na
Fold Change in LD50 of Venom/Toxin									
B. caeruleus venom	5	3.7	nd	2.2	1.8 (ns)	3.9	5.7	5.2	1.5 (ns)
B. multicinctus venom	3.0	2.6	1.7 (ns)	7.4	4.6	6.0	11	3.8	2.0 (ns)
α-Bungarotoxin	1.4 (ns)	0.9 (ns)	nd	nd	1.0 (ns)	1.2 (ns)	1.1 (ns)	nd	nd
β-Bungarotoxin	17	3.8	nd	1.9	4.0	3.9	8.6	5.0	nd
C. durissus venom	nd	nd	nd	nd	nd	nd	nd	nd	nd
Crotoxin	nd	nd	1.6 (ns)	nd	nd	nd	nd	nd	nd
O. scutellatus venom	0.9	nd	3.5	nd	nd	nd	nd	nd	nd
Taipoxin	nd	nd	4.0	nd	nd	nd	nd	nd	nd

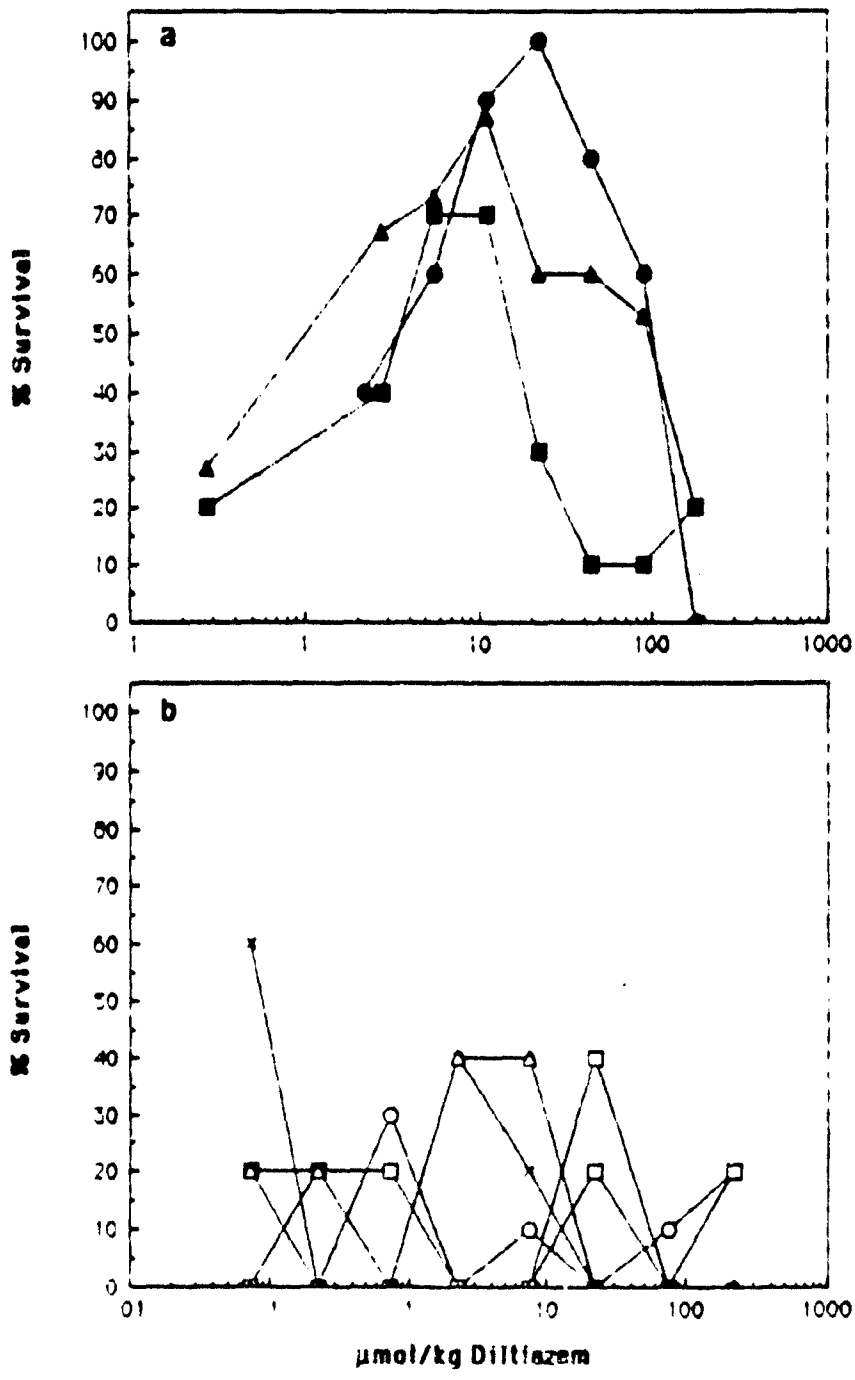
Drug	Charge (a) pH 7.2	IC50 ACh (b) Trnsprt (µM)	MW (Daltons)	Solubility (c) (M)	Ki PLA2 (µM)	Reference, Ki
Chloroquine	1.9	0.5	320	0.48	165	AUTHI and TRAYNOR, 1979
Chlorpromazine	1.0	3.0	319	1.4	27	JAIN and JAHAGIRDAR, 1985
Dexamethasone	-1.5		392	0.23	1	PILTCH et al., 1989
Diltiazem	1.0		415	1.1	100	BROEKMEIER, et al., 1985
Nicergoline	0.9		484	0.00026	0.1	NIKOLOV and KOBUROVA, 1984
Nifedipine	0.05		346	0.00036	50	CHANG, et al., 1987
Piracetam	0.0		142	3.5	20	NIKOLOV and KOBUROVA, 1984
Primaquine	1.9		259	0.15	17	AUTHI and TRAYNOR, 1979
Quinacrine	1.8	0.4	400	0.019	400	BROEKMEIER, et al., 1985
Reserpine	0.2	8.0	608	0.000082		
Verapamil	1.0		455	0.0041	200	BROEKMEIER, et al., 1985
Vesamicol	1.0	0.04	259	0.011		
Vesamicol 72		0.1				

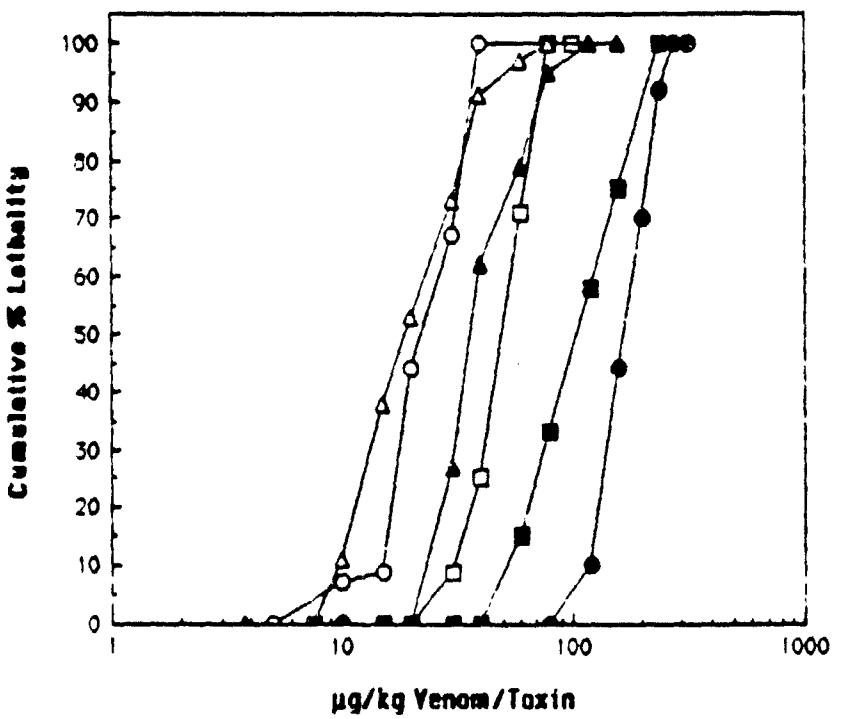
Venom/Toxin	Molecular Weight	Solubility	Charge pH 7.2
B. caeruleus	0.10	-0.02	0.62 (a)
B. multicinctus	0.10	-0.25	0.76 (b)
β-Bungarotoxin	-0.02	-0.18	0.71 (c)

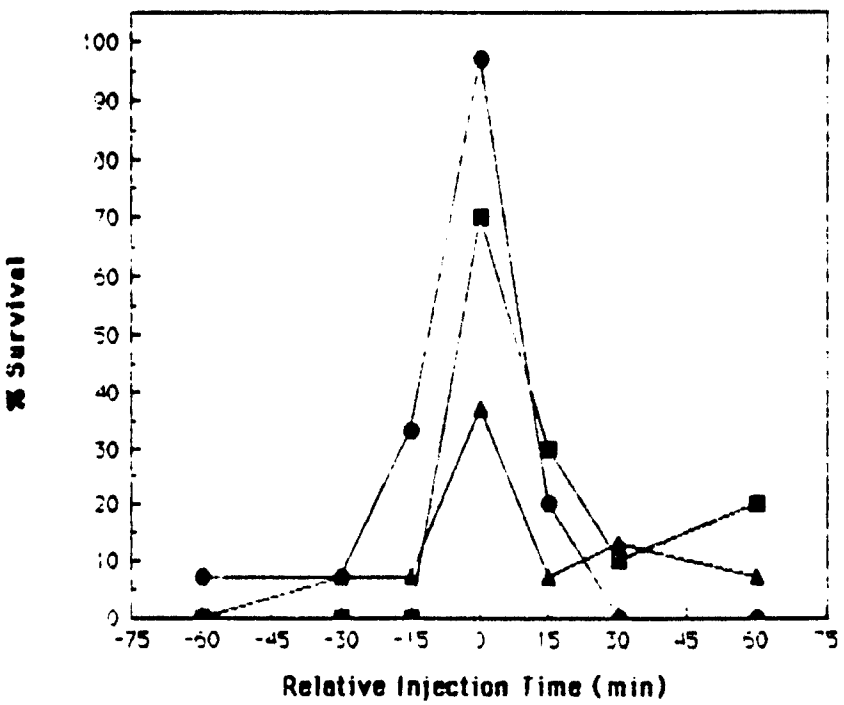

a
b
% Survival
% Survival
100
90
80
70
60
50
40
30
20
10
0
.01
.1
1
10
100
1000
10000
µmol/kg Dexamethasone


Cumulative % Lethality
100
90
80
70
60
50
40
30
20
10
0
.1
1
10
100
1000
µg/kg Venom or Toxin

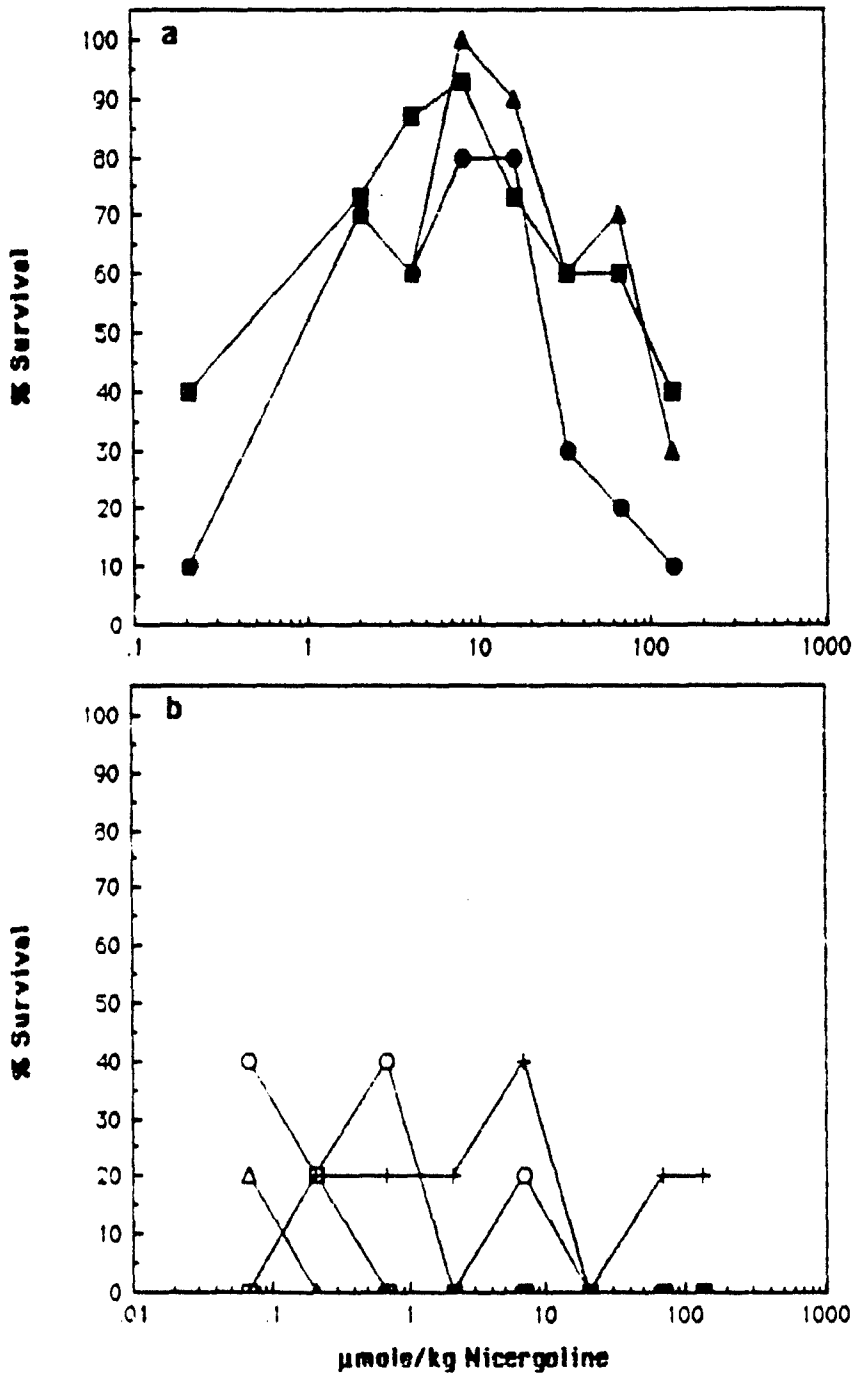

% Survival
100
90
80
70
60
50
40
30
20
10
0
-75
-60
-45
-30
-15
0
15
30
45
60
75
Relative Injection Time (min)

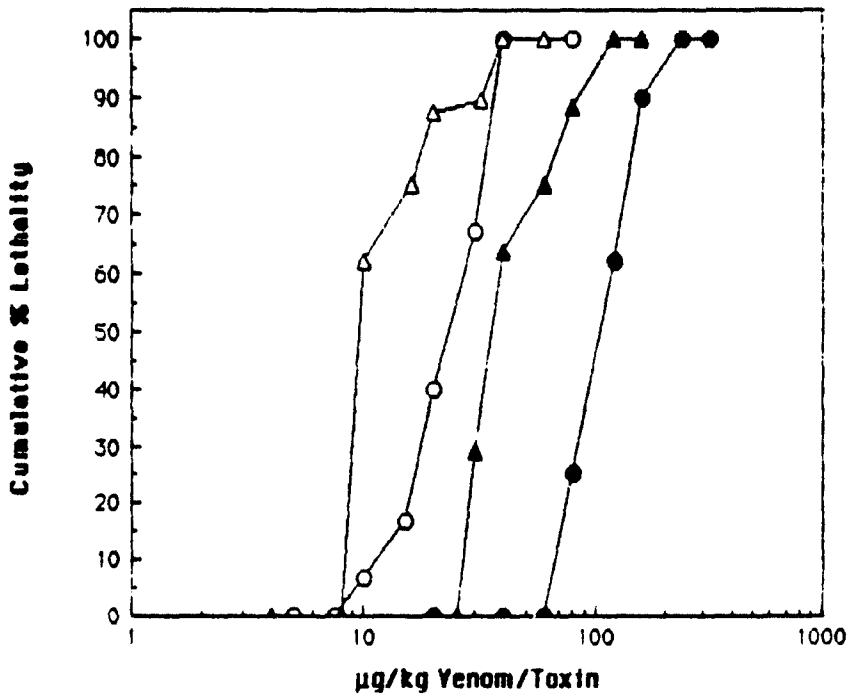

a
b
% Survival
% Survival
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
µmol/kg Diltiazem

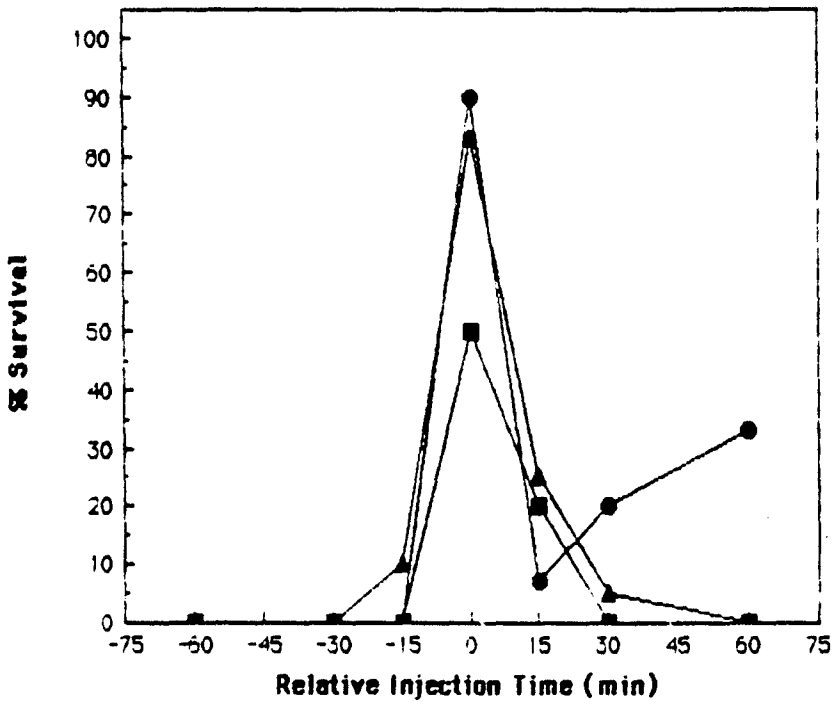

Cumulative % Lethality
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
µg/kg Venom/Toxin

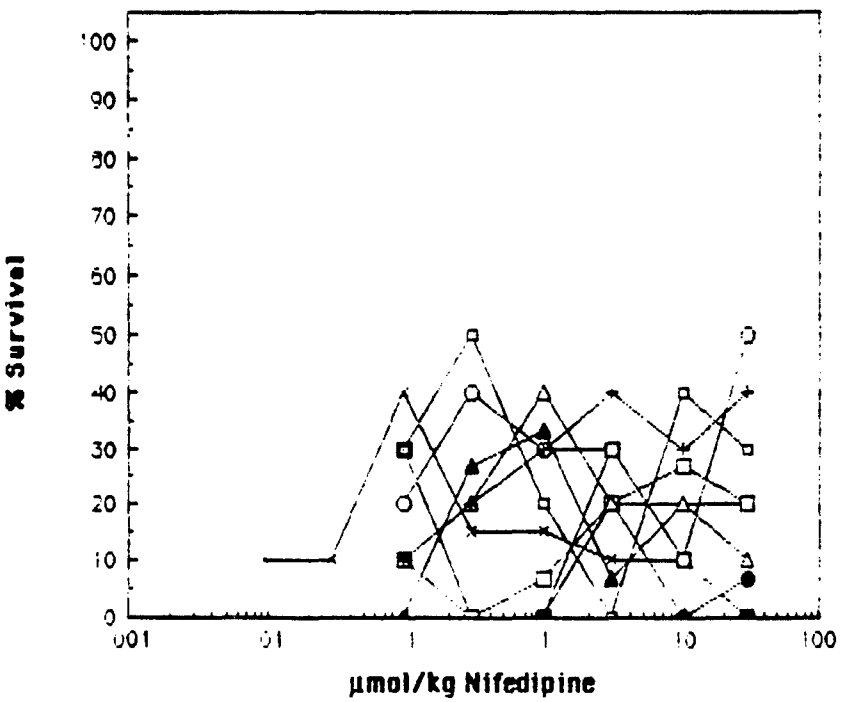

% Survival
100
90
80
70
60
50
40
30
20
10
0
-75
-60
-45
-30
-15
0
15
30
45
60
75
Relative Injection Time (min)

F

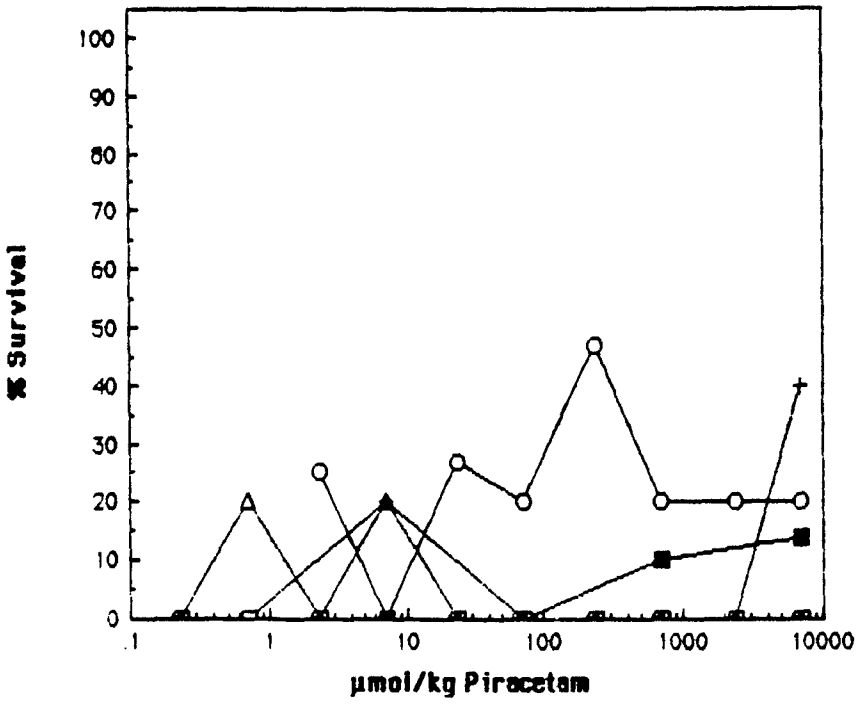

a
b
% Survival
% Survival
100
90
80
70
60
50
40
30
20
10
0
.01
.1
1
10
100
1000
µmole/kg Nicergoline

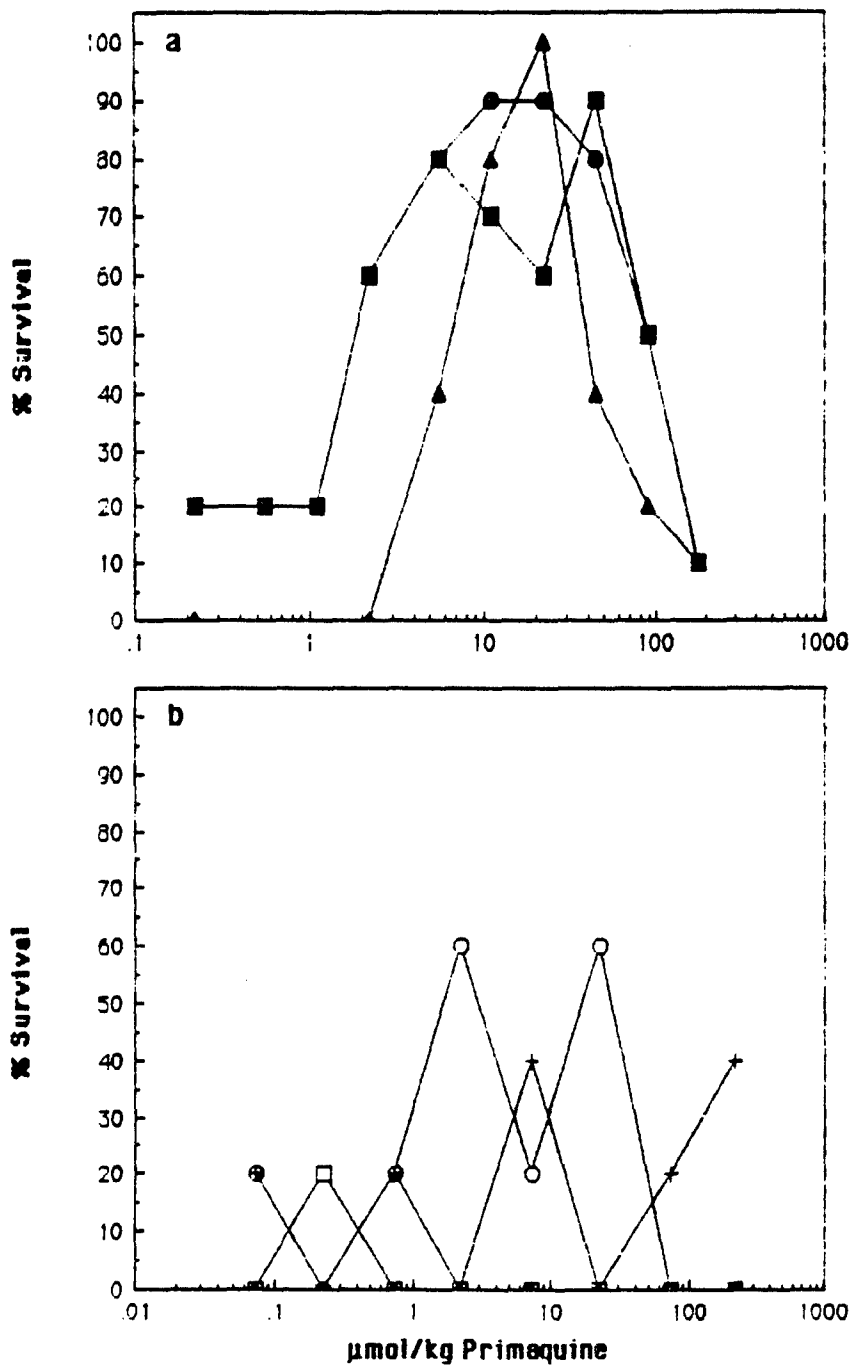

Cumulative % Lethality
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
µg/kg Venom/Toxin

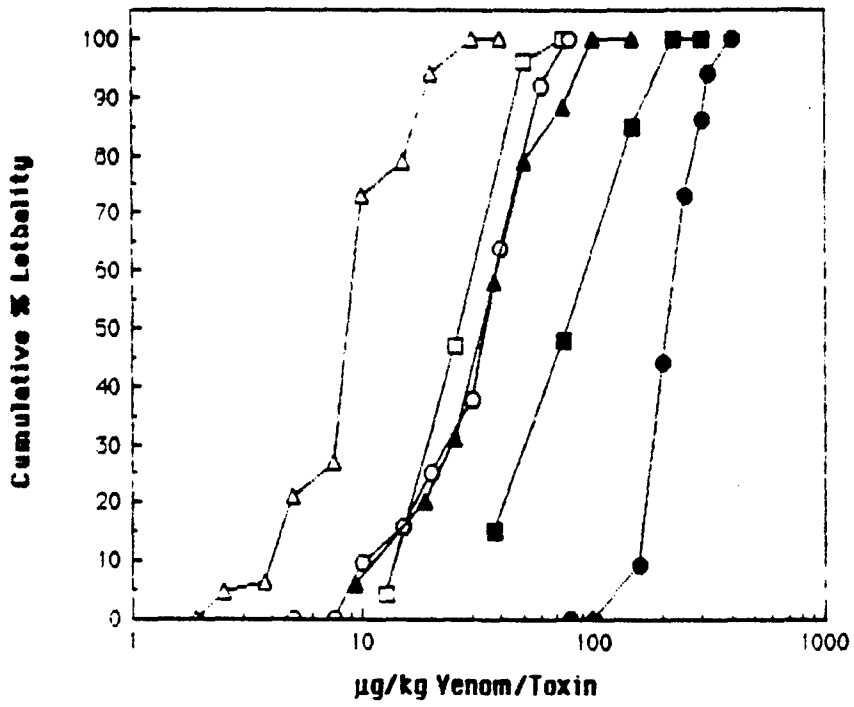

% Survival
100
90
80
70
60
50
40
30
20
10
0
-75
-60
-45
-30
-15
0
15
30
45
60
75
Relative Injection Time (min)

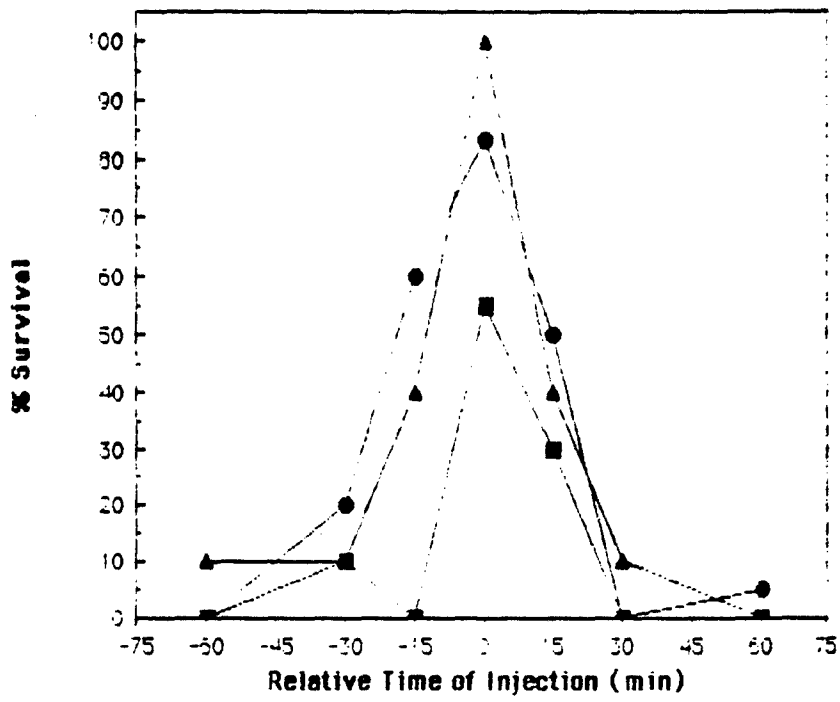

% Survival
100
90
80
70
60
50
40
30
20
10
0
001
01
1
1
10
100
µmol/kg Nifedipine

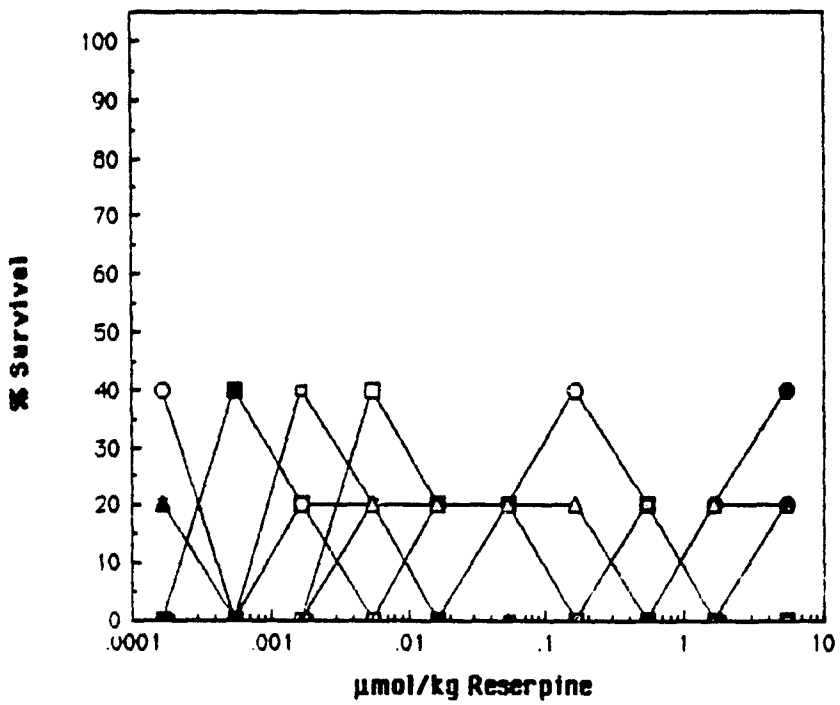
F

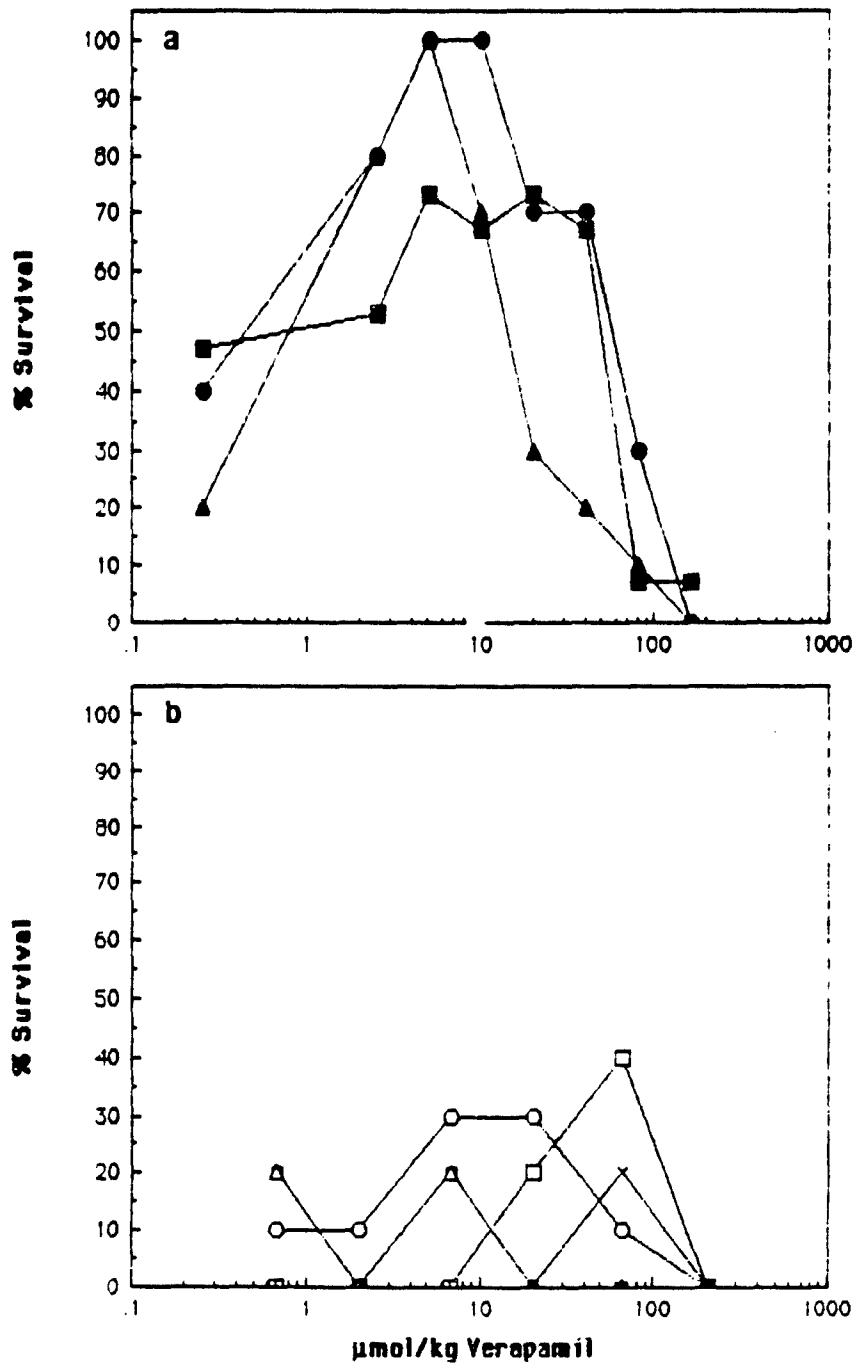

% Survival
100
90
80
70
60
50
40
30
20
10
0
.1
1
10
100
1000
10000
µmol/kg Piracetam

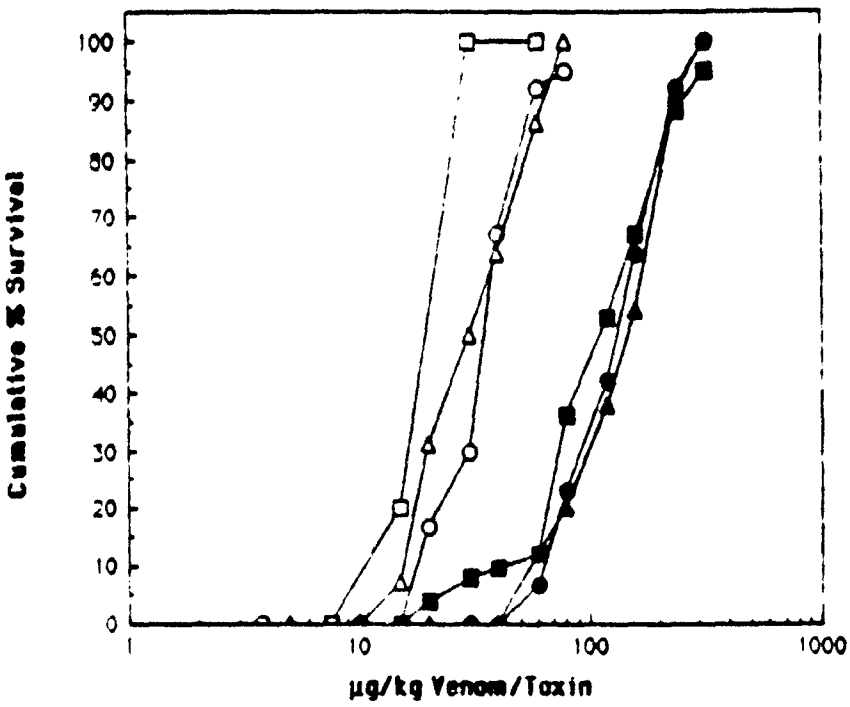

a
b
% Survival
% Survival
µmol/kg Primaquine
0
10
20
30
40
50
60
70
80
90
100
.01
.1
1
10
100
1000

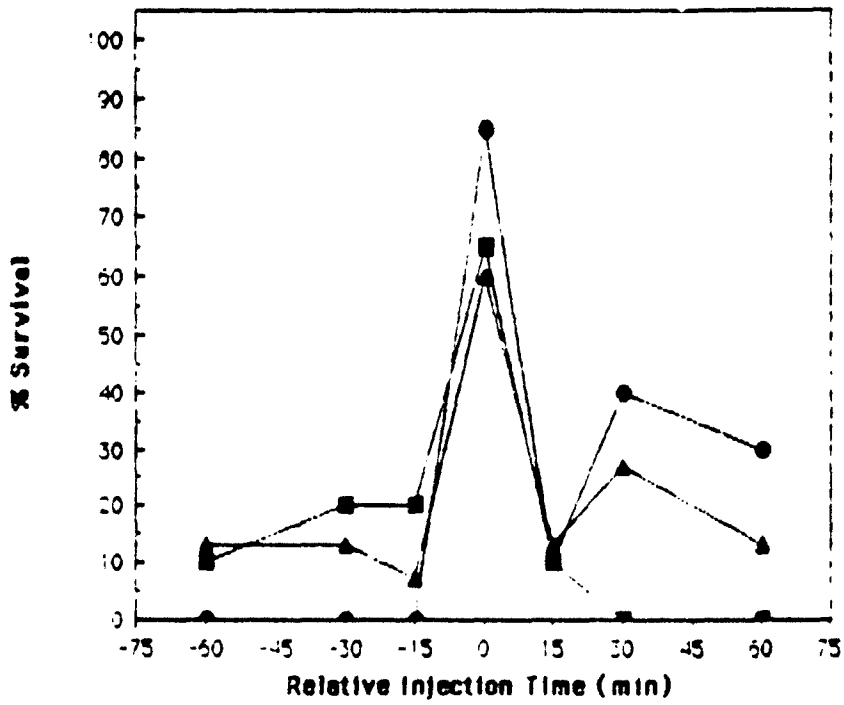

Cumulative % Letbality
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
µg/kg Venom/Toxin

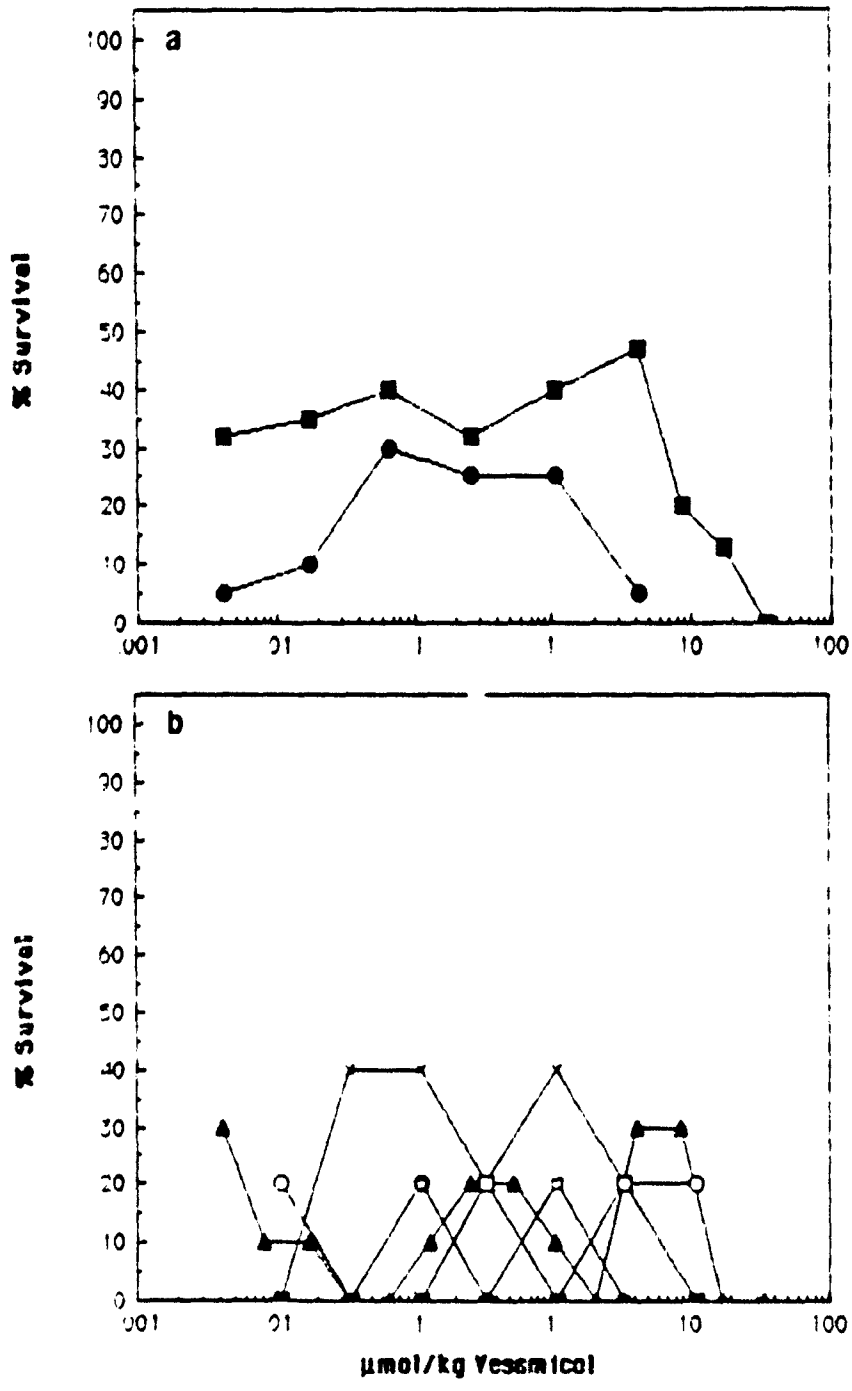

% Survival
100
90
80
70
60
50
40
30
20
10
0
-75
-60
-45
-30
-15
0
15
30
45
60
75
Relative Time of Injection (min)

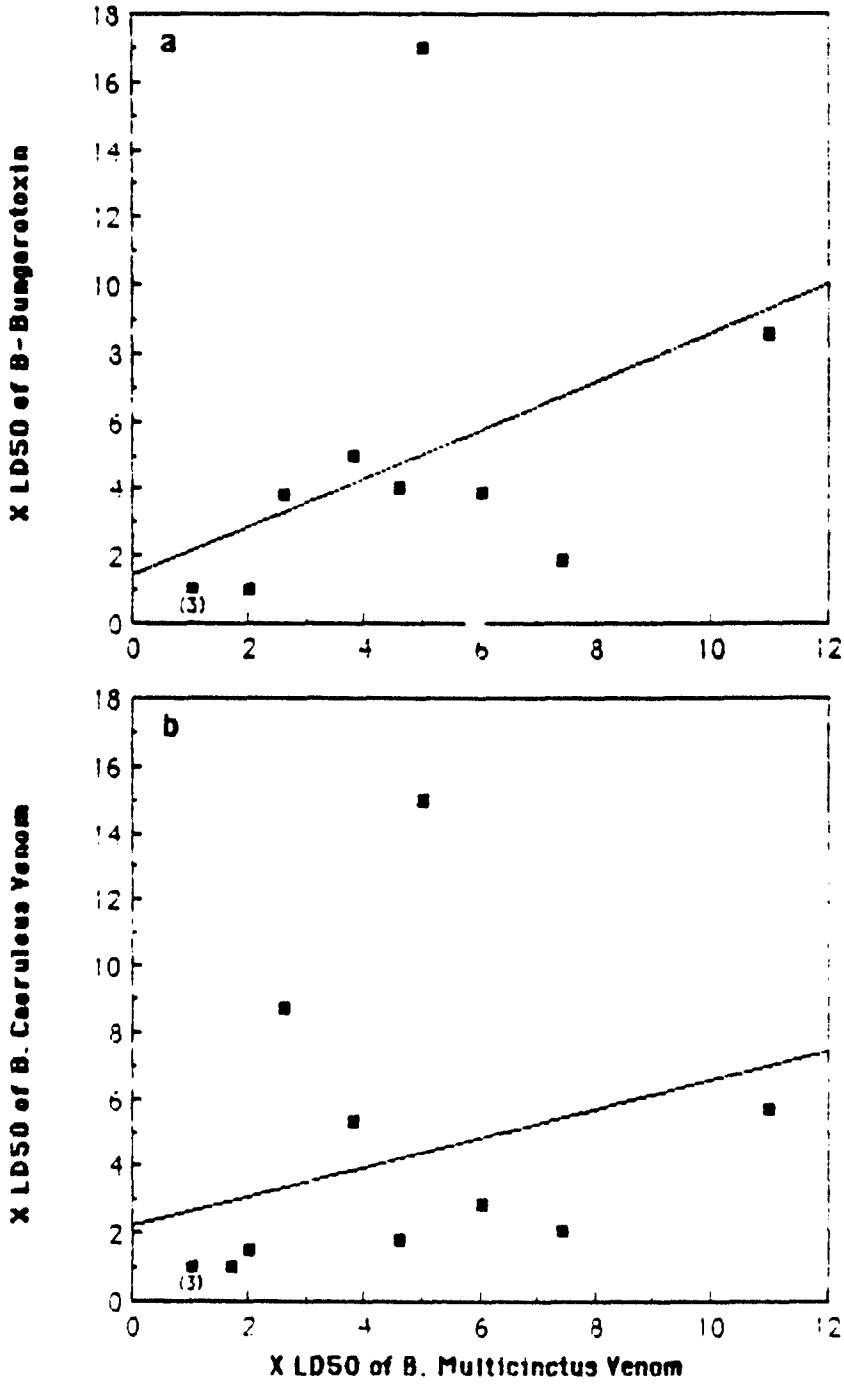

% Survival
100
90
80
70
60
50
40
30
20
10
0
.0001
.001
.01
.1
1
10
µmol/kg Reserpine

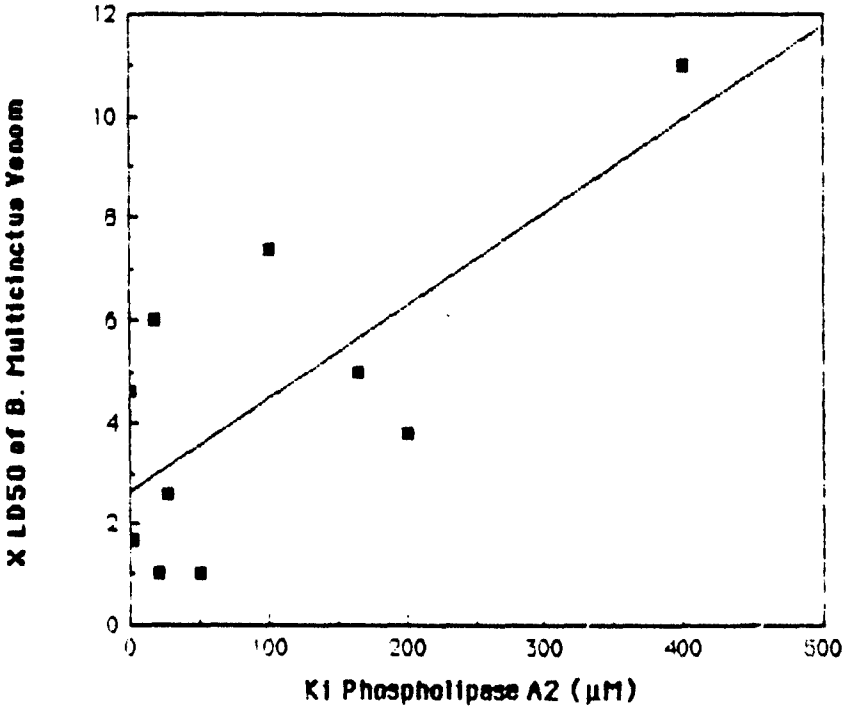

a
b
% Survival
% Survival
100
90
80
70
60
50
40
30
20
10
0
.1
1
10
100
1000
µmol/kg Verapamil

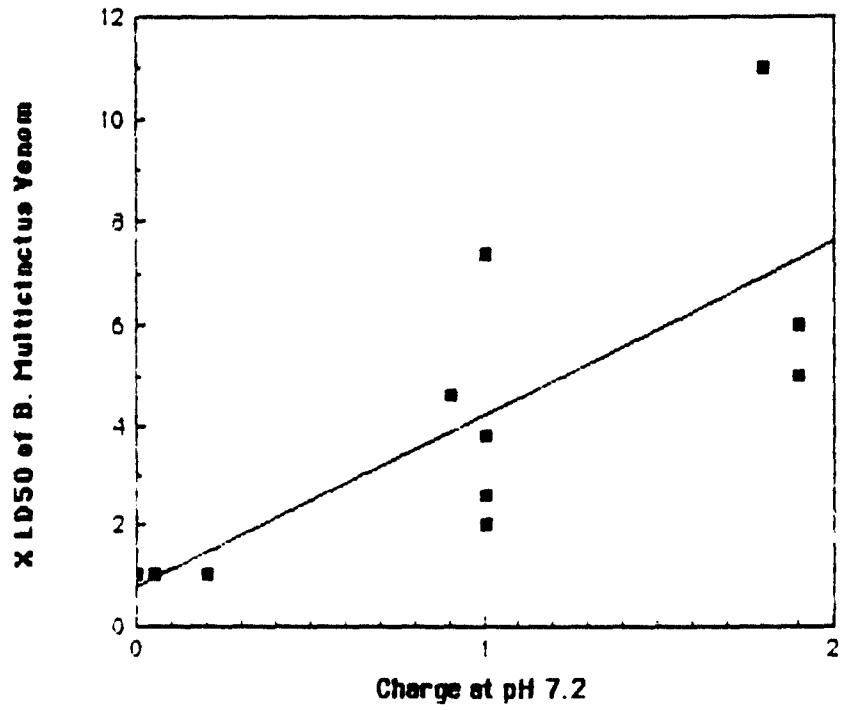

Cumulative % Survival
100
90
80
70
60
50
40
30
20
10
0
1
10
100
1000
µg/kg Venom/Toxin


% Survival
100
90
80
70
60
50
40
30
20
10
0
-75
-60
-45
-30
-15
0
15
30
45
60
75
Relative Injection Time (min)


a
b
% Survival
% Survival
100
90
80
70
60
50
40
30
20
10
0
.001
.01
1
1
10
100
µmol/kg Vesamicol


a
b
X LD50 of B-Bungerotoxin
X LD50 of B. Caeruleus Venom
X LD50 of B. Multicinctus Venom
(3)
(3)
0
2
4
6
8
10
12
14
16
18


X LD50 of B. Multicinctus Venom
0
2
4
6
8
10
12
0
100
200
300
400
500
Ki Phospholipase A2 (µM)


X LD50 of B. Multicinctus Venom
12
10
8
6
4
2
0
0
1
2
Charge at pH 7.2